VOLUME 41 OCTOBER, 1955 NUMBER 5

The Journal of PARASITOLOGY

OFFICIAL ORGAN OF THE
AMERICAN SOCIETY OF PARASITOLOGISTS
FOUNDED BY HENRY BALDWIN WARD

EDITORIAL COMMITTEE

GEORGE R. LA RUE, CHAIRMAN
CLAY G. HUFF
CORNELIUS B. PHILIP
EVERETT E. LUND, ASSISTING THE COMMITTEE

EDITORIAL BOARD

WILLIAM BALAMUTH
ELON E. BYRD
RAYMOND M. CABLE
G. ROBERT COATNEY
WILLIAM W. CORT
CHAUNCEY G. GOODCHILD
GLEN M. KOHLS
ALLEN McINTOSH
LOUIS J. OLIVIER
NORMAN R. STOLL
HORACE W. STUNKARD
PAUL E. THOMPSON

OFFICERS OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

President: CLAY G. HUFF
President-elect: ELOISE B. CRAM
Vice-president: GILBERT F. OTTO
Secretary: ARTHUR C. WALTON, Knox College, Galesburg, Illinois.
Treasurer: ROBERT M. STABLER, Colorado College, Colorado Springs, Colorado.

Council members ex officio: GEORGE R. LA RUE, *Chairman, Editorial Committee.* WILFORD S. BAILEY, *Custodian, Back Issues.* NORMAN R. STOLL, *Custodian, Endowment Fund.*

Council Members at Large: RAYMOND M. CABLE, AUREL O. FOSTER, ALLEN MCINTOSH, DONALD V. MOORE, ELON E. BYRD, CHESTER A. HERRICK, LUTHER O. NOLF, LESLIE A. STAUBER.

Persons interested in any branch of parasitology may apply for membership in the American Society of Parasitologists. Those interested in membership are invited to write to the Secretary who will furnish membership application blanks. The annual dues for the Society ($6.00) includes a subscription to THE JOURNAL OF PARASITOLOGY.

THE JOURNAL OF PARASITOLOGY

THE JOURNAL OF PARASITOLOGY, published bi-monthly, is the official organ of the American Society of Parasitologists and as such it publishes the program of the annual scientific meeting, abstracts of the papers presented there, and minutes of the business meetings of the Society.

THE JOURNAL OF PARASITOLOGY is a medium for the publication of the results of research in the field of animal parasitology. Manuscripts dealing with subjects in this field will be considered for publication, and accepted manuscripts will be published in order of receipt. A manuscript is accepted with the understanding that it is to be published exclusively in the Journal unless otherwise arranged in advance.

Articles submitted for publication may be sent to any member of the Editorial Committee or Editorial Board. Responsibility for acceptance of papers is divided in the Editorial Committee for the fields of helminthology, protozoology and entomology. Correspondence concerning the disposition or revision of manuscripts should be addressed to George R. La Rue, Chairman of the Editorial Committee, Parasite Research Laboratories, A.R.S., Beltsville, Maryland.

Manuscripts submitted should be typewritten, double or triple spaced, with wide margins, and with tables, graphs, and illustrations in finished form for transmission to the printer. Title, author, institution, etc., go on a separate sheet. Each table requires a separate sheet. Footnotes are not encouraged. Such material can usually be embodied in the text. If footnotes are used, they should be numbered consecutively and typed on a sheet separate from the text. Authors are urged to emphasize simplicity and clearness in tables. When feasible, sharp, contrasty photographic reproductions of large plates and graphs should be submitted in place of the originals. In preparing plates care should be given to the effective arrangement of figures, to economy of space, and to the amount of reduction required. Figures should be arranged within an area five by eight inches, or multiples thereof. Legends for figures should be typed on a sheet, separate from the text, and containing no illustrations. Greek characters and symbols should be avoided, where possible, since they require hand composition, thus increasing the cost of publication.

The bibliography, double or triple spaced, should contain only those references actually cited in the text, and its style should conform to bibliographies in recent issues of this Journal. The Index Catalogue of Medical and Veterinary Zoology should be used as a guide for abbreviating the journals cited.

Since it is the desire of the Editorial Committee to publish all acceptable manuscripts submitted within the limited space available, authors are urged to present their results briefly, avoiding tables where possible. However, acceptability of a paper will be determined not by its length but by the amount and character of new information included and the form in which it is presented. Long articles, and those with an unusual number of tables and plates, can be published only with the financial cooperation of the author.

Reprints may be purchased at cost from the printer if they are ordered when galley proof is returned. A blank form accompanies the proof for this purpose.

Claim for copies lost in the mails must be received by the Treasurer within sixty days following the mailing date. Delivery overseas cannot be guaranteed and must be at the subscriber's risk. Changes of address must be received by the Treasurer at least two weeks prior to the date of issue.

The subscription price for the Journal is $7.50 a volume (Foreign $8.00).

Correspondence concerning subscriptions and changes of address should be addressed to the Treasurer.

Correspondence concerning orders for back issues should be addressed to the Custodian, Wilford S. Bailey, Alabama Polytechnic Institute, Auburn, Alabama.

Entered as second-class matter December 21, 1932, at the post office at Lancaster, Pa., under the Act of March 3, 1879.

The Journal of Parasitology

Volume 41 OCTOBER, 1955 Number 5

THE MORPHOLOGY AND LIFE HISTORY OF *AMPHIACANTHA OVALIS* AND *A. ATTENUATA*, TWO NEW HAPLOSPORIDIAN PARASITES OF GREGARINES*

JOSEPH W. STUBBLEFIELD
Department of Biology, Compton College

INTRODUCTION

Amphiacantha ovalis sp. nov., and *A. attenuata* sp. nov. are intracellular parasites of the gregarine *Lecudina* sp. Mingazinni. The genus *Lecudina* Mingazinni is considered to be the same as *Ophioidina* Mingazinni and *Doliocystis* Leger by Kamm (1922), Bhatia (1930), and Ganapatti and Aiyar (1937). The gregarine host of these new hyperparasites is itself parasitic in the intestine of the polychaet worms *Lumbrineris latreilli* Audouin and Edwards (see Hartman, 1944, p. 158), and *L. zonâta* (Johnson) (see Hartman, 1944, pp. 146–147).

The genus *Amphiacantha* belongs to the METCHNIKOVELLIDAE established by Caullery and Mesnil (1914) for a number of similar hyperparasites of gregarines. They classified members of this family according to the character of the cyst which I have now, for the first time, identified as a gametocyst. Gametocysts of the two new species show considerable similarity to that of *Amphiacantha longa* found by Caullery and Mesnil in the same or in a closely related species of gregarine from *Lumbrineris latreilli* and accordingly they have been placed in the same genus. Ganapatti and Aiyar (1937) mentioned a Metchnikovellid from a closely related species of gregarine, *Lecudina brasili*. They considered this to represent the genus *Metchnikovella*, but no description was given and the possibility is suggested that it may also represent the genus *Amphiacantha* in view of the near-identity of annelid and gregarine hosts.

The widely separate localities from which these gregarines harboring hyperparasites of the genus *Amphiacantha* have been taken suggest the possibility that the same or closely allied gregarines with the same genus of hyperparasites may be found in intermediate localities.

Caullery and Mesnil (1919) published a review of the METCHNIKOVELLIDAE, listing 13 species in 3 genera. Since that time, 5 new species of the family have been described and one of these has been referred to an additional genus (*Caullerycetta*). Data regarding all the known species of the family are presented in Table I, based upon that of Caullery and Mesnil (1919) but enlarged to include species since described. Caullery (1953, p. 931) has recently suggested that known

Received for publication, May 25, 1954.

* The researches leading to this publication were submitted in partial fulfillment of the degree of Doctor of Philosophy in Zoology, to the University of California, Berkeley.

TABLE I.—*List of described species of* METCHNIKOVELLIDAE

METCHNIKOVELLIDAE	Gregarine host	Annelid host	Dimensions of cyst in microns	Number of gametocytes in cyst
Metchnikovella spionis C. & M., 1897	*Polyrhabdina brasili*	*Spio martinensis*	20–40 by 4	16
M. brasili C. & M., 1919	*Ibid.*	*Ibid.*	10 by 15	12
M. incurvata C. & M., 1914	*Polyrhabdina pygospionis*	*Pygospio seticornis*	18–22 by 4	16
M. oviformis C. & M., 1914	*Ibid.*	*Ibid.*	14 by 6.5	8
M. nereidis C. & M., 1914	*Lecudina pellucida* or related species	*Platynereis dumerilii*	10–12 by 4	8
M. hessei C. & M., 1914	*Monocystis* (? *Selenidium*) *mitis*	*Fredericia polychaeta*	(?) 10–12 by 3–4	12
M. selenidii Averintsev, 1908	*Selenidium* sp.	*Ophelia limacina*	(?) 15–29 by 7	(?)
M. minima C. & M., 1914	*Selenidium cirratuli*	*Audouinia tentaculata*	8.7 by 4.3	(?)
M. legeri C. & M., 1914	*Sycia inopinata*	*Ibid.*	20–30 by 5.5–7	32
M. claperedei C. & M., 1914	flattened diamond-shaped species	*Phyllodoce* sp.	27 by 4.5	(?)
M. shereshevskaiae nom. nov. = *Microsporidyopsis nereidis* Shereshevskaia, 1924	*Doliocystis* sp.	*Nereis parallelogramma*	(?)	(?)
Metchnikovella caulleryi M. & R., 1931	*Polyrhabdina polydorae*	*Polydora flava*	85–100 by 2.5–3	20–30
M. polydorae Reichenow, 1932	*Selenidium* (? *cruzi*)	*Polydora* sp.	10–14 by 3	12–16
Amphiamblys capitellidis C. & M., 1897	*Ancora* sp.	*Capitellidis giardi*	35–40 by 2.5	32
A. capitellae C. & M., 1914		*Capitella capitata*	50–60 by 3	(?)
A. ancorae Reichenow, 1932	*Ancora sagittata*	*Ibid.*	50–60 by 4.5	(?)
Amphiacantha longa C. & M., 1914	*Lecudina* (*Ophioidina*) sp.	*Lumbrineris latreilli*	70–80 by 4.5	(?)–100
A. attenuata sp. nov.	*Ibid.*	*Ibid.* and also *L zonata*	45–80 by 4.5–6	32–60
A. ovalis sp. nov.	*Ibid.*	*Ibid.*	40–95 by 6–8	14–50
Caulleryetta mesnili Dogiel, 1922	*Selenidium* sp.	*Travisia forbesi*	(?)	8–12

members of the family should all be referred to the type genus *Mechnikovella*, and that other genera (*Amphiamblys*, *Amphiacantha*) should be reduced to the status of subgenera, but it is my considered opinion that any effort to clarify the taxonomic status of species within the family should await further study.

Caullery and Mesnil (1897, 1914, 1919) published a few notes on the life history of the species which they studied. Averintsev (1908) also recorded observations on the development of *Metchnikovella selenidii*. Although the findings of each of these investigators agreed in essential points, all were incomplete, and heretofore no integrated account of the succession of stages in the life history has been presented. The stage which I have now identified as the isogamete was thought by these earlier observers to be an infective spore, which having gained entrance to the host, produced a plasmodium by growth and nuclear division. This plasmodium varied in different species, being markedly elongated in some, while in others it was more in the form of a compact or of a branched plate. Uninucleate early spores were believed to be formed by division of the plasmodium, and the cyst wall was supposed later to be formed around groups of these early spores, the latter being simultaneously transformed into the infective stages.

Schereschevskaia (1924) described the cyst as being secreted about two conjugating uninucleate cells of a size considerably greater than the so-called early spores of Caullery and Mesnil. A sexual basis for the conjugation was merely inferred, and it is possible that dividing plasmodia and early binucleate cysts were confused. Schereschevskaia took the hypothetical mode of cyst formation as described by Caullery and Mesnil as established fact, and was thus led to believe that the species which she investigated did not belong to the METCHNIKOVELLIDAE but constituted a connecting link between that group and the MICROSPORIDIA where she placed the new species. It is my opinion that the species, described by Schereschevskaia, is properly a member of the genus *Metchnikovella*, and its name is here changed from *Microsporidyopsis nereidis*, proposed by her, to *Metchnikovella schereschevskaiae*. The change in specific trivial designation is proposed since the original name, *nereidis*, is already in use in the genus *Metchnikovella*.

As a result of the fragmentary knowledge of the morphology and life history of representatives, the systematic position of the METCHNIKOVELLIDAE in the PROTISTA has been uncertain. Very recently, Caullery (1953) repeated a frequently expressed view that this family is probably related to the fungi. Further comment on the taxonomy of the group will be deferred until after presentation of observations on the new species.

The author wishes to acknowledge indebtness to the late Professors Charles A. Kofoid and Harold Kirby, Jr. for valuable aid in the conduct of research herein reported, and to Professor Gordon H. Ball for advice and assistance with the manuscript. Responsibility for all observations and conclusions is, however, solely that of the author.

MATERIALS AND METHODS

All specimens of *Lumbrineris* used in this investigation were collected from one locality at Duxbury Reef, two miles north of Bolinas, Marin County, California. Worms were collected at all seasons of the year and maintained in the laboratory for several weeks without noticeable variation in the incidence of parasitism.

Identifying features of certain stages of the hyperparasite could be recognized in fresh living preparations, and some effort was made to observe the progress of the stages in sealed smears. Although gametocysts and free gametocytes retain their normal appearance in such preparations for as long as two weeks, continued development appears to be inhibited. Since it was impossible to follow the sequence of stages by direct observation, it was necessary to rely upon the method of searching out the various stages and recognizing a morphological continuity between them.

A few microtome sections were prepared, but smears proved much more convenient, and were used almost exclusively. Best fixation was obtained with Schaudinn's and Brazil's fluids, especially when employed with 15–20% glacial acetic acid at 50–60° C. Such severe fixation appears to be necessary to insure adequate penetration of the gregarine host and contained gametocysts.

Smears were stained with Heidenhain's iron hematoxylin, and also by the iron-hematoxylin method of Craig and Wilson (1937). The latter method proved particularly convenient for the rapid processing of large numbers of smears without the necessity of a critical appraisal of destaining. The method of Heidenhain, however, produced superior cytological differentiation.

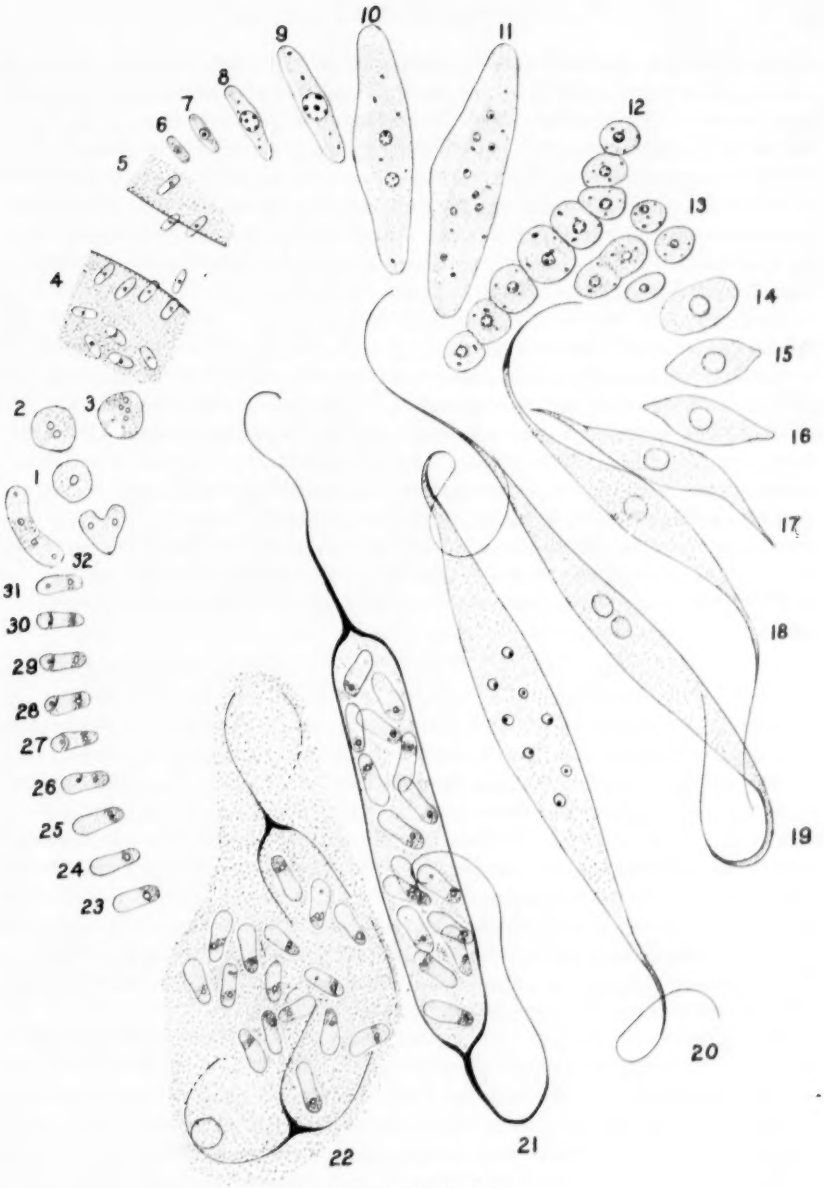
DESCRIPTION OF THE SUCCESSIVE STAGES IN THE LIFE-CYCLE OF AMPHIACANTHA OVALIS

(Plates I and II, Figs. 1–35)

The nature and sequence of stages in the life-cycle of *Amphiacantha ovalis* are diagrammatically represented in Text Figure A. Only the stages from gametocysts through the early sporonts of the two new species have been distinguished from each other during this investigation, and other stages are presumed to be closely similar in both species.

Sporozoites—A supposedly motile stage (Pl. I, Figs. 1–5) has been frequently encountered in gregarines harboring one or both new species of *Amphiacantha*. No similar stage has been described previously in the METCHNIKOVELLIDAE. I have not observed this stage in living preparations, hence have had no opportunity to observe them in actual movement. The view that they are motile is based solely on the regularity of alignment which they display within the gregarine cytoplasm. At one end of the sporozoite a clear oval vesicle is to be observed, while the cytoplasm at the opposite end is regularly stained more deeply. The nucleus is more or less centrally placed. In a gregarine host carrying 200 or more sporozoites, an estimated minimum of 80 percent of them are oriented with great regularity with their darker stained ends pressed against, and distributed all around, the cell membrane of the host, and having the end with the clear vesicle projecting toward the center of the host cytoplasm (Figs. A, 4–5). I am convinced that such dramatic regularity of alignment within the host would be probable only for a motile cell, that it gives evidence that these cells are in process of leaving the host, and that this is a stage which does in fact leave one host and actively seeks another.

The usual length of the sporozoites (Pl. I, Figs. 1–5) is about 2.5 microns but the sporozoites grade imperceptibly in size and structure into a series of growth stages. When heavily stained, these bodies show a striking resemblance to certain bacterial spores due to the presence of the clear vesicle previously mentioned. However, when more faintly stained, the clear vesicle is less well defined and a dark-



EXPLANATION OF TEXT FIGURE A

1. Zygote with synkaryon.
2. Binucleate sporont.
3. Tetranucleate sporont.
4–5. Sporozoites in the host cytoplasm, and illustrating the probable means of transfer from one host to another.
6–11. Growth stages developed from sporozoites.
12–13. Conclusion of schizogony to produce trophozoites.
14–21. Development of the gametocyst.
22. Disintegration of the gametocyst within the host cytoplasm.
23–31. Maturation of gametocytes.
32. Cytoplasmic fusion of isogametes to produce the prozygote.

stained granule is observed near the center of the cell; it is presumed to be the nucleus and in some instances a faint nuclear membrane may be observed surrounding an unstained intermediate zone. Moreover, a nuclear membrane is clearly evident in the growth stages (Pl. I, Figs. 10–11) which follow. There is some resemblance of the terminal non-staining vesicle to the polar capsule present in the spores of certain MICROSPORIDIA, but no polar filament has been observed. The darker staining material at one end of the cell may be further concentrated at the tip of the cell, may form an equatorial band, or may be so arranged as to resemble an hourglass with the nucleus centrally placed (Pl. I, Figs. 1–5).

Since the exact manner of sporozoite formation has not been observed, and since these cells have not been reported by other investigators of the METCHNIKOVELLIDAE, a question might be raised as to whether they belong to another organism. In support of the view that these all belong to one species, the following facts may be cited: (1) Growth of sporozoites may be traced to trophozoites which seem clearly to represent *Amphiacantha*. (2) Sporozoites, like certain other specific stages in this life cycle, are not encountered frequently; and might easily be missed unless a prolonged search is carried out. (3) Sporozoites have very rarely, if ever, been encountered in the absence of identifiable stages of *Amphiacantha* within the same individual gregarine or within other gregarines parasitizing the same annelid. (4) No other stage of hyperparasite appears to possess attributes adaptive to dissemination.

Growth of Sporozoites—Wherever found, some sporozoites have generally commenced to grow, and stages up to 10 microns in length may be encountered alongside unchanged sporozoites. Cells not unlike the sporozoite in size and conformation show a different distribution of stainable material within the cytoplasm, a change which is believed to mark the initiation of growth. Stainable substance may be found localized at either end (Pl. I, Fig. 8) or about the nucleus in the center of the cell. Often a delicate membrane may be observed surrounding a clear zone characteristically present about the nuclear granule, and the nucleus soon assumes a vesicular character. In no one of these is it still possible to distinguish the clear vesicle so evident at one end of the sporozoite. Although a few young growing individuals may be found around the host periphery, generally there is no tendency to a regular alignment in that position as with the preceding stage, and it is inferred that motility has been lost.

In the sporozoite, the nucleus is located in the zone of darker-staining cytoplasm, usually slightly removed from the center of the cell. Later its position is more central (Pl. I, Fig. 6). Possibly the nucleus is carried along in the redistribution of stainable material and simultaneous disappearance of the clear vesicle (Pl. I, Fig. 7) from one end. Later, the nuclear granule enlarges somewhat and is located at the center of a clear zone (Pl. I, Fig. 9), an arrangement which suggests that a nuclear membrane is present in every case, but it is not generally visible until a length of 4 microns has been attained (Pl. I, Fig. 10). Simultaneously, the cell thickens appreciably and a longitudinal zone of clear cytoplasm becomes visible all the way along one side. The nucleus occupies the entire central portion of the cell, traversing the dark zone on one side and the light zone on the other.

In cells of a length of 4–5 microns, the nucleus occupies about one-half the volume of the cell (Pl. I, Fig. 10) being equal to its greatest breadth and one-half

its length. By this stage, or shortly thereafter, the nuclear chromatin is dispersed in 1 to 5, or occasionally more, heavily-staining granules situated around the inner surface of the nuclear membrane or free in the nuclear sap. Moreover, some chromatin is present more or less evenly distributed around the inner surface of the nuclear membrane, in an amount which is less when the granules are not so evident. In later trophozoites, the distribution of chromatin is highly variable.

With the above changes in distribution of cytoplasmic contents, and in position and character of the nucleus, there is established a structural arrangement which changes but little during subsequent growth. Growth of the nucleus does not, however, keep pace with that of the cytoplasm. For the most part, these stages are widest in the middle, and taper gradually toward the rounded ends (Pl. I, Fig. 12), although some individuals are more cylindrical in form and show a restricted oval enlargement about the nucleus. When such semi-cylindrical, elongated growth stages are abundant within a single host, they may be so clumped together as to resemble the mycelia of certain fungi, a fact which contributed to the conclusion reached by some observers that the METCHNIKOVELLIDAE are related to the fungi.

Generally, the uninucleate trophozoite does not exceed a length of 25–30 microns, but some individuals measuring up to 40 microns in length have a giant nucleus as much as 6 microns in diameter. Such cells are not unlike the smaller ones, measuring 13 microns (Pl. I, Fig. 12) save for their greater size and relatively smaller nuclei. The trophozoites and later plasmodia are characterized by a granular cytoplasm with irregular areas of thickening. Most cytoplasmic granules stain but faintly while others, presumed to be volutin, stain very deeply.

Schizogony to produce trophozoites—Nuclear division and subsequent plasmotomy occur with marked irregularity. I have studied many specimens in which nuclei elongated into an oval form or dumbbell in shape, appear to be dividing, but the details of the process remain obscure. The number of nuclei produced prior to plasmotomy is evidently variable, and it seems probable that fission may occur at any time after the first nuclear division. The occasional occurrence of one, two, or three gametocysts in a given gregarine serves to emphasize the irregularity of plasmotomy, and the possible degeneration of the products thereof. However, as many as 8 nuclei occur in some plasmodia. A plasmodium having 5 nuclei undergoing division into two daughter plasmodia is shown in Plate I, Fig. 16. Note that the nuclei in this individual are of markedly different sizes, a circumstance often encountered. Presumably smaller nuclei are those recently formed; the larger ones older. In other cases (Pl. I, Fig. 15) division of the plasmodium occurs simultaneously along the full length of the organism. Plasmodial division may be accomplished by the formation of broad constrictions (Pl. I, Fig. 16) or by the growth of a membrane across the cells where a narrow indentation has previously appeared (Pl. I, Fig. 15).

Since it is not difficult to recognize a succession of trophic forms leading from sporozoites to elongated plasmodia as just described, I have been led to conclude that such plasmodia represent a first generation. Plasmodia do not always occur in this elongated form, however, but are sometimes in the form of rounded or branched plates. Moreover, the uninucleate products of plasmotomy, perhaps in association with undivided plasmodia, are sometimes massed together in an arrangement suggesting a cluster of grapes, the superficial units of which are generally

uninucleate but in which as many as 4 nuclei may occasionally be observed. Such variability suggests that a second generation of plasmodia occurs.

Even in cases where cytoplasmic division progresses at a uniform rate throughout the plasmodium, the uninucleate products are markedly variable in size (Pl. I, Fig. 17). These cells when approximately spherical in form, may vary from 3.2 to as much as 9 microns in diameter; when oval they are as much as 10 microns in length. Nuclei in these stages have chromatin either in the form of large discrete granules, or more or less evenly distributed on the inner surface of the nuclear membrane.

Gametocyst development—Earliest pre-gametocyst stages are distinguished from preceding stages by a more elongated form, a larger, more faintly staining nucleus, the infrequent occurrence of volutin, which is never present in large granules, and generally by a more even distribution of stainable material within the cytoplasm. Soon thereafter the rudiments of the terminal filaments appear (Fig. A, 15–17).

The general course of later stages in gametocyst development is depicted in Figure A (18–21) which shows that growth continues until gametocytes are differentiated. Observations suggest that there is a progressive elongation of the filaments during cyst growth. There can be little doubt that the filaments are composed of the same material as the cyst wall, and in all probability both are simultaneously formed, just as both disappear together after dehiscence of gametocytes.

Specimens are observed in which the only traces of terminal filaments are the attenuated ends of the growing organism but these soon appear to be drawn out producing filaments tapering gradually toward the tip (Fig. A, 16–19). It appears that the entire organism, save perhaps the very thin tips of the filaments, is initially one continuous protoplasmic mass, enlarged centrally and gradually attenuated toward each end (Pl. I, Fig. 19). Hardening of the filaments evidently prevents further expansion in that region while the central portion of the organism continues to grow until it is of uniform breadth from filament base to filament base, thus establishing the contours of the mature cyst. There is a progressive thickening and increase in stainability of the cyst wall throughout development. The mature cyst is evidently rigid, and although it may sometimes be wrinkled, it maintains its original form until final disintegration in the host cytoplasm (Fig. A, 22).

Rapid disintegration and resorption of the cyst is evidenced by the fact that gregarines having gametocytes free in the cytoplasm rarely show any trace of the cyst wall which previously surrounded them, a fact which led most earlier observers to conclude that the gametocytes precede the cyst in the cycle. Schereshevskaia (1924), as mentioned above, did observe the dehiscence of gametocytes from the mature cyst of *Metchnikovella* (= *Microsporidyopsis*) *schereschevskaiae*.

Cytoplasm of later gametocysts is usually more or less alveolar in appearance. Small slightly-stainable granules are arranged about the alveoli and irregular, often radiating, thickenings may be present. For the most part, nuclei cannot be distinguished in the developing gametocysts after the appearance of the terminal filaments, but stages having such filaments and as many as 8 nuclei have been found (Pl. I, Fig. 19). Study of a very large number of developing cysts indicates that the cyst wall is decidedly impermeable to reagents once the filament stage has been attained. Often the interior of cysts is but slightly stained while other parts of the preparation are very dark, and traces of picric acid may continue to stain cyst

contents long after it has been removed completely from the remainder of a preparation. Permeability appears to increase to some extent once gametocytes have been differentiated.

Formation and structure of gametocytes—In gametocysts whose thickened walls and broad ends indicate approaching maturity, faint lines suggesting the delineation of future gametocytes may be observed. Gametocyte nuclei generally are not visible until these cells are completely differentiated, probably because of their small size and the difficulty of obtaining satisfactory staining of cyst contents. Irregular masses of stainable material, presumed to be residual cytoplasm, may remain after formation of gametocytes. An estimated 97 percent of mature cysts (Pl. I, Fig. 20) contain from 14 to 32 gametocytes. Cysts containing more than 40 are decidedly rare, but a single cyst containing 50 such cells was observed.

Gametocytes from the time of their formation until fertilization occurs, are oval in form, and are somewhat pear-shaped, being a little narrower at one end with lateral indentation. Most of them measure 7–8.5 microns in length. In the youngest gametocytes, a single nucleus is situated not more than one-third of the distance from the narrow end, usually a little to one side of the longitudinal axis, or even against the cell membrane. When favorably stained, an accumulation of chromatic material may be observed in the cytoplasm at the narrow end of the cell where it forms a sort of polar cap arranged about the interior of the cell membrane. The chromatic material, which is usually smooth and devoid of visible structure, but sometimes granular, may be localized at the narrow end of the cell, at one side of that end in an irregular fashion, or it may form a transverse band at the level of the nucleus.

Gametocytes, both within the mature gametocyst (Pl. I, Fig. 20) and after dehiscence, (Pl. II, Figs. 21–31) are surrounded by a clear zone which is notably lacking around other stages. Residual stainable material within the mature gametocyst is never close against the cell membranes of gametocytes, and the latter cells are clearly delineated by their unstained halo. The latter is of fairly uniform thickness, but its outer limits are notably vague thus enhancing the suggestion of a halo. I have concluded that the gametocytes are possibly surrounded by a layer of non-staining, jelly-like material since alternate explanations, such as shrinkage of gametocyte or thinning of host cytoplasm, fail to account for the absence of similar effects around other stages.

Possible Meiosis—In later gametocytes of *Amphiacantha ovalis*, there occur two nuclear divisions which produce polar nuclei destined to disintegrate. The history of these nuclei, particularly their disintegration, strongly suggests that they are actually formed by a meiotic process, even though it has not been possible to confirm this by evidence of chromosomal reduction *per se*. Minute size of gametocyte nuclei has made it impossible to follow more than the gross features of these divisions. The first maturation division may be complete and the second commenced prior to dehiscence (Pl. I, Fig. 20), but in other cases it appears that cyst rupture precedes nuclear division in the gamete, it is possible to follow the process more readily in extra-cyst cells (Pl. II, Figs. 22–31).

Prior to each division there is an enlargement of the nucleus which elongates into an oval form transversely placed within the cell (Pl. II, Figs. 22, 28). The next recognizable stage shows two equal nuclei situated side by side as though

formed by constriction of the oval nucleus (Pl. II, Figs. 29, 31). Following both the first and second of two such divisions, one daughter nucleus shows unmistakable signs of degeneration before the two have separated (Pl. II, Fig. 23). The degenerating daughter nucleus, hereinafter referred to as the *polar nucleus,* is soon located farther and farther toward the broad end of the cell, and stainability progressively decreases until it becomes very faint and resembles a vacuole, although a centrally-placed granule may persist for a long time (Pl. II, Figs. 25–28).

After the polar nucleus has migrated as far as the middle of the cell, a minute cleft is frequently visible extending from the cell surface inward to this moribund nucleus. Such a cleft is not clearly visible except when the cell is seen in a suitable position (Pl. 2, Figs. 25, 26, 27), and it is most clearly to be seen in quite deeply stained cells. In some individuals, presumed to represent a later stage, (Pl. II, Figs. 27, 29), the cleft clearly separates from the parent cell, a small cell containing the polar nucleus. I have concluded this is possibly analogous to the polar cells of classical oogenesis in metazoa. Evidently the polar cell never does separate from the functional gamete but remains in a cup-like depression. Possible stages in a progressive degeneration of these polar cells have been observed and Pl. II, Fig. 29, shows a gametocyte in which the nucleus of the shrunken polar cell has largely disappeared. Observations of shrinking polar cells suggest that the latter are gradually resorbed or crowded by the parent cell as degeneration progresses.

The sequence of certain events in maturation, as in other phases of the life-cycle, appears to be variable. Failure to observe a cleft indicates that a second nuclear division may occur before separation of the polar cell (Pl. II, Fig. 31) and also the possibility that in some instances a cleft may never be formed. The minute size of the nucleus, the inability to resolve the details of nuclear changes during division, and the absence of discernible difference between the early gametocyte and mature gamete, leave no means for distinguishing a first nuclear division from the second, save in cases where the latter occurs before evidence of the former has disappeared (Pl. II, Figs. 29–31). For these reasons I have not been able to ascertain whether a second polar cell may ever be formed.

Fertilization. Cytoplasmic fusion to form a prozygote may occur between gametes placed end to end (Pl. II, Fig. 32) or side by side, but in all cases, where recognizable, the more narrow, darker staining ends are in apposition. Characteristically 2 gametes fuse, but I have observed a few cases where 3 gametes appear to have fused. Significant confirmation of the sequence of stages is provided by the fact that gametes may fuse prior to disappearance of polar nuclei (Pl. II, Fig. 32). The shape of the prozygote, and in some cases of the zygote (Pl. II, Fig. 33) as well, is dependent upon the placement of the fusing cells, some being elongate, others of a more compact and ovoid form. All such cells appear to round up prior to nuclear division in the sporont.

Sporogony—I have found a new binucleate and quadrinucleate cells (Pl. II, Figs. 34–35) surrounded by the same type of halo-like, non-staining zone, and in the presence of gametes, prozygotes, and zygotes, thus giving strong presumptive evidence that these constitute a development series. I have concluded that these cells do, in fact, follow the zygote in the life cycle, and that they are sporonts. I have not, however, been able to discover later stages in which they are presumed to form sporozoites.

DESCRIPTION OF STAGES IN THE LIFE CYCLE OF *AMPHIACANTHA ATTENUATA*

(Plate II, Figs. 36–45)

The name *Amphiacantha attenuata* has been given to the second species of metchnikovellid parasitic in the gregarines harbored by *Lumbrineris latreilli* and *L. zonata* because of the more attenuated form of its gametocyst as contrasted to the more ovoid type of *A. ovalis*. These two species have been found with equal frequency and occasionally both parasitize the same individual gregarine, but more frequently they occur in different gregarines within the same annelid host. Throughout much of the life cycle, the two species have not been distinguished. Sporozoites, growth stages, plasmodia, and early gametocysts are indistinguishable.

At the time gametocysts of *A. attenuata* begin to mature, they still retain the form characteristic of both species in earlier stages, namely, a central region, broader than the remainder of the organism and a gradually increasing attenuation toward each end. This form, as previously described, is considerably modified in maturing cysts of *A. ovalis*. Growth and expansion of the cyst of *A. attenuata* follows a different course and instead of expanding to a uniform breadth between the bases of the terminal filaments, it continues to be attenuated from the center distally, possibly because of an earlier hardening of the cyst wall. That part of the cyst which encloses the developing gametocytes is clearly marked off from the filaments by an accumulation, at the junction, of material which appears to be identical to that of which the cyst wall is composed, forming a plug-like structure of variable appearance (Pl. II, fig. 36). One or two large vesicles, or numerous smaller ones producing an alveolar appearance may occur. Caullery and Mesnil (1914, 1919) reported similar plugs at the ends of cysts in some species which they described. Shereshevskaia (1924), and Reichenow (1932), working with *Metchnikovella* (= *Microsporidyopsis*) *schereschevskaiae* and *M. polydorae* respectively, figured curious examples of a similar structure in which one end of the cyst appeared markedly thicker than the other.

Nuclei are as difficult to trace through the period of cyst development in *Amphiacantha attenuata* as in *A. ovalis*, and the great majority of cysts do not show nuclei until after the formation of gametocytes.

Gametocytes of *A. attenuata* (Pl. II, Figs. 37–42) are spherical to subspherical in contrast to the more ovoid form of those of *A. ovalis*, and may number from 30 to 60, most frequently from 45 to 50, within a cyst. Gametocyte cytoplasm shows areas of increased stainability, but these are of less regular location than in *A. ovalis*. An irregular mass of more deeply staining cytoplasm frequently occurs in the vicinity of the nucleus, but it may occur in any position, even lying across the cell from the nucleus. Gametocyte nuclei are similiar to those of *A. ovalis* save for their somewhat smaller size, and may be found pressed against the cell membrane (Pl. II, Fig. 37).

The gametocyte nucleus undergoes 2 meiotic divisions (Pl. II, Figs. 39–42) which are essentially similiar to those previously described in *A. ovalis*. Prozygotes, (Pl. II, Fig. 43); zygotes (Pl. II, Fig 44); and sporonts (Pl. II, Fig. 45) are all similiar to those of *A. ovalis*, save for an average smaller size. They have been distinguished from *A. ovalis* by their simultaneous occurrence along with the distinctly different gametes.

Family METCHNIKOVELLIDAE Caullery and Mesnil, 1914, Compt. Rend. Soc. Biol., Paris. **77**: 527.

Diagnosis.—Haplosporidia parasitic in gregarines from marine annelids; characterized by motile sporozoites without spore walls, and with elongated plasmodia developed from sporozoites; gametocysts simple (*Metchnikovella minima* Caullery and Mesnil, 1914) or elaborate (*Amphiacantha* C. and M., 1914), number of gametocytes produced within gametocysts variable or constant in different species, varying in number from 8 (*Metchnikovella oviformis* C. and M., 1914), to 100 (*Amphiacantha longa* C. and M., 1914); 3 successive multiplication periods in life cycle, viz., schizogony to produce trophozoites, schizogony to produce gametocytes, and sporogony, combined with ability to repeat the entire cycle within the same host rendering ultimate destruction of host highly probable.

Type genus—*Metchnikovella* Caullery and Mesnil, 1897.

Genus *Amphiacantha* Caullery and Mesnil, 1914, Compt. Rend. Soc. Biol., Paris. **77**: 527.

Diagnosis.—METCHNIKOVELLIDAE having gametocyst drawn out into a short (*A. longa*) or a long (*A. ovalis* sp. nov., and *A. attenuata* sp. nov.) filament at each end, gametocysts gradually attenuated from the center to tips of filaments (*A. longa, A. attenuata*) or of approximately equal breadth between bases of filaments *A. ovalis*), number of gametocytes produced ranging from 14 (*A. ovalis*) to 100 (*A. longa*).

Type species: A. longa C. and M., 1914.

Amphiacantha ovalis sp. nov.
(Plates I–II, Figs. 1–35)

Diagnosis.—Mature gametocysts of approximately equal breadth between bases of terminal filaments, latter tapered gradually from bases to distal tips and marked off from remainder of cyst by their lesser breadth and by slight thickening of cyst wall at their bases; length of mature gametocysts from 40 to 95 microns between filament bases with each filament equalling three-fourths to one times this length; gametocytes ovoid, often somewhat pear-shaped with greatest diameter 5 to 9.5 microns, numbering from 14 to 50 in different cysts with 50 percent having 16; maturation charcterized by two divisions, possibly reductional, of gametocyte nucleus and formation of one (possibly two) polar cell; motile sporozoites ovoid, averaging 2.5 microns in length with one end dark staining and opposite end marked by presence of non-staining vesicle; early growth stages of sporozoite with large central nucleus and two lateral bands in cytoplasm, one darker staining and wider containing nucleus; schizogony to produce trophozoites quite irregular both in time of occurrence and in size of uninucleate cells produced.

Type host: Lecudina sp.

Amphiacantha attenuata sp. nov.
(Plate II, Figs. 36–45)

Diagnosis.—Mature gametocysts gradually attenuated from center outward to tips of terminal filaments, latter marked off from remainder of cyst by plugs of same material as cyst wall; mature gametocysts from 45–80 microns between plugs, with each filament equal to one-half to two-thirds this length; spherical to subspherical gametocytes from 3 to 4 microns in diameter, numbering from 32 to 60 in different cysts; other stages indistinguishable from corresponding stages of *A. ovalis*.

Type host.—Lecudina sp.

DISCUSSION

Host-Parasite Relations—The obvious impoverishment of gregarine cytoplasm, as a direct result of crowding, constitutes the only direct evidence of destructive action of *Amphiacantha* upon its host observed by me. Also, no apparent basis has been found for concluding that the occasional degenerate stage of *Amphiacantha* is a product of counteraction by the gregarine host. It is concluded, therefore, that continued growth and reproduction of the hyperparasite of necessity leads to destruction of its host. Gametocytes and spores represent a climax of such growth and reproduction and, by the time these stages have been reached, extensive destruction of host cytoplasm is generally evidenced by crowding as well as by thinning and loss of stainability.

The life cycle—I have concluded that the sporozoite of *Amphiacantha* is both naked and motile, although the evidence of motility is admittedly circumstantial and inconclusive. There is an obvious resemblance of the clear vesicle at the posterior

end of the sporozoite of *Amphiacantha* to the polar vesicle of various microsporidia of the family NOSEMITIDAE (*vide* Kudo, 1946, fig. 257), but I find no other basis for homologizing the two, since the sporozoite of *Amphiacantha* is naked and without polar filament. It is possible, however, that spores of certain species which have been placed in the MICROSPORIDIA may be in fact both naked and without a polar filament.

From a structural viewpoint, the elaborate gametocyst is certainly a unique feature in the two new species, though this structure appears better developed here than in any other representative of the family. No particular utility for this cyst, nor for the terminal filaments, are suggested. To assume that the cyst is protective for the gametocytes, necessitates the parallel assumption that the parasite is more vulnerable at this stage than in other stages which lack a protective covering. Perhaps this structure may represent a holdover from a phylogenetically earlier circumstance of life when it did in fact serve a useful protective role.

The process of maturation as described for *A. ovalis* is unique with reference to the separation of a polar cell from the gametocyte. I have not been able to discover any reference to a comparable phenomenon in the entire realm of the PROTISTA. Moreover, it differs from the formation of polar bodies in METAZOA in these respects: (1) the polar cell in *Amphiacantha* does not separate from the gametocyte immediately, but only after the nucleus has migrated approximately half the length of the cell and after it has undergone marked decrease in stainability; (2) separation of the polar cell is not related to a division spindle; (3) separation of the polar cell is accomplished by a furrow cutting across the cell, not by the uplifting of an elevation on the surface of the cell as typical of the metazoon oocyte; (4) the polar cell remains closely attached to the functional gametocyte and appears to be resorbed by the latter.

Paedogamy, reported in several representatives of the HAPLOSPORIDIA (see Naville, 1931), is an admitted possibility in *Amphiacantha*, though I have found no evidence that it occurs. Meiosis appears to be initiated regularly within the gametocyst and might conceivably be complete in some individuals prior to dehiscence of gametocytes, but I have not observed any case of fusion of gametes within the gametocyst. Nevertheless, paedogamy is not excluded as a possibility in this genus, since gametes from the same cyst may later fuse in the host cytoplasm.

The production of nuclei destined to disintegrate and the formation of a polar cell, seem to indicate the occurrence of pregametic reduction in *Amphiacantha* in spite of the fact that it has not been possible to observe an actual reduction in the number of chromosomes. There is little evidence from studies on other HAPLOSPORIDIA which might indicate the point at which reduction occurs.

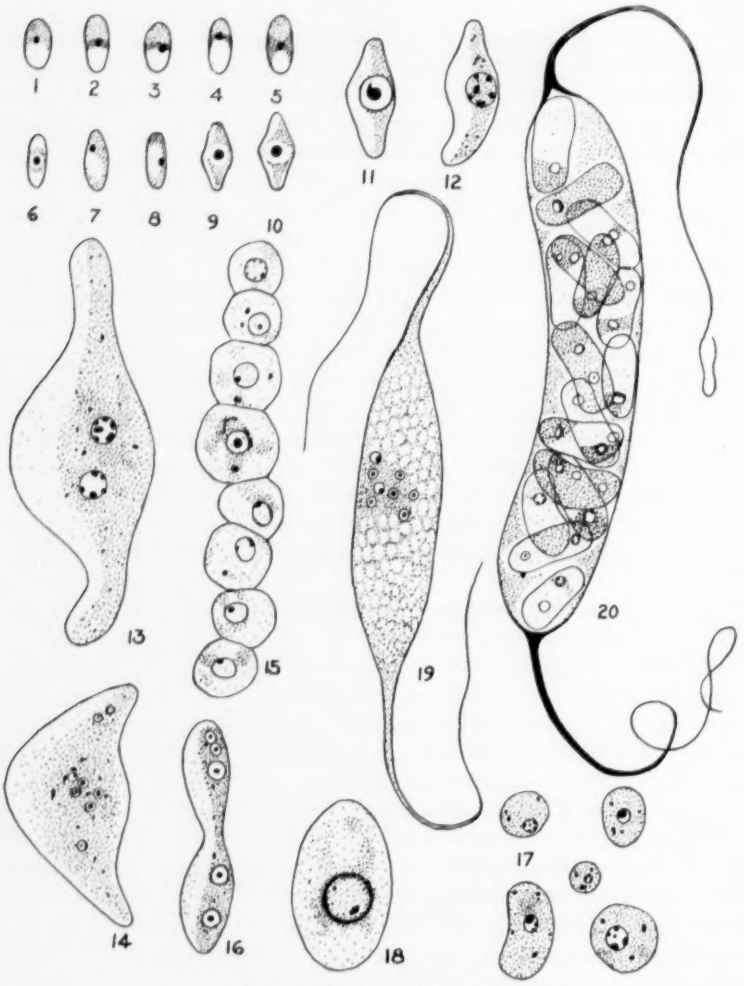
Systematic position of the METCHNIKOVELLIDAE—Since the earliest descriptions, the systematic position of the organisms now grouped in the METCHNIKOVELLIDAE has been uncertain. Caullery and Mesnil (1897) at first made no attempt to place them in the system of PROTISTA. Later (1905) they placed them with the HAPLOSPORIDIA and suggested a possible relationship to the fungi. Averintsev (1908), and Reichenow, (in Doflein 1929, p. 1137) also placed the family in the HAPLOSPORIDIA, but Reichenow later (1932, p. IIg 20) did not assign them to any particular group and merely suggested that they probably belong to the lower fungi.

On the basis of observations herein reported, it seems clearly proper to discount

Explanation of Plates

All drawings were made with the aid of a camera lucida from smears fixed with Schaudinn's fluid with 15–20% glacial acid at a temperature of 50–60° C., and stained with iron hematoxylin. Unless otherwise stated the magnification of all figures is 3200×.

Plate I



Amphiacantha ovalis, (figs. 1–20)

Figs. 1–5. Sporozoites, before the initiation of growth, showing the centrally placed nuclear granule and the differential staining of the two ends.

any special affinity of these organisms with the fungi. The sequence of vegetative and sexual stages follows the pattern of the Haplosporidia as summarized by Naville (1931, p. 74), and accordingly I favor placing the Metchnikovellidae in this order.

SUMMARY

1. This report deals with the systematic position and life history of *Amphiacantha ovalis* sp. nov., and *A. attenuata* sp. nov. which are parasites of gregarines.

2. In the life history of these parasites, schizonts develop from sporozoites, gametes are formed within a cyst, and fertilization to form sporonts follows excystment.

3. Gametogenesis involves two nuclear divisions, possibly reductional, and one daughter nucleus from each division degenerates. In *A. ovalis* a unique polar cell encloses the degenerating nucleus.

4. Destruction of the gregarine host by growth and division of the hyperparasites appears inevitable.

LITERATURE CITED

AVERINTSEV, A. V. 1908 Studien über parasitische Protozoen, I–VII. Trudy Imp. Obsh. Estestovis., Vypusk 2: Otdiel Zool. i. Fiziol. **38:** 1–139 (In Russian with German summary appended).

BHATIA, B. L. 1930 Synopsis of the genera and classification of haplocyte gregarines. Parasitology **22:** 156–167.

CAULLERY, M. 1953. Protozoaires: rhizopodes, actinopodes, sporozoaires, cnidosporidies. Traité Zool. (Grassé) **1:** (2), 1160 pp.

CAULLERY, M. AND MESNIL, F. 1897 Sur un type nouveau (*Metchnikovella* n.g.) d'organismes parasites des grégarines. Compt. Rend. Soc. Biol. Paris **49:** 960–962.

———1905 Recherches sur les haplosporidies. Arch. Zool. Exper. et Gen. 4S **4:** 101–181.

———1914 Sur les Metchnikovellidae et autres protistes parasites des grégarines d'annélides. Compt. Rend. Soc. Biol. Paris **77:** 527–532.

———1919 Metchnikovellidae et autres protistes parasites des grégarines d'annélides. Ann. Inst. Pasteur, Paris **33:** 209–240.

CRAIG, R. AND WILSON, C. 1937 The use of buffered solutions in staining: theory and practice. Stain Tech. **12:** 99–109.

DOFLEIN, F. 1929 Lehrbuch der Protozoenkunde, neubearbeitet von Dr. Edouard Reichenow 5th ed., 1262 pp. (Jena.)

DOGIEL, V. A. 1922 Sur un nouveau genre de Metchnikovellidae. Ann. Inst. Pasteur, Paris **36:** 574–577.

FIG. 6. Early stage in the growth of a sporozoite showing the nuclear membrane, the centrally placed nuclear granule, and dark staining cytoplasm massed about the nucleus.

FIG. 7. Initiation of growth marked by movement of dark staining material which obliterates the clear vesicle at the posterior end.

FIG. 8. Somewhat later stage, after the movement of cytoplasmic materials is complete.

FIGS. 9–10. Later growth stages of sporozoites, showing a clear zone around the centrally located nuclear granule, but no visible nuclear membrane. Differential staining between the two sides of the organism is visible, a character which persists during later growth stages and into the period of schizogony.

FIG. 11. Later growth stage showing the breakup of the nuclear granule.

FIG. 12. Later growth stage, showing chromatin in the form of granules located for the most part around the inner surface of the nuclear membrane. Volutin granules are present. 1600×.

FIG. 13. A binucleate stage developed from a sporozoite. 1600×.

FIG. 14. An 8-nucleate stage, just prior to cytoplasmic division concluding schizogony to produce trophozoites. 1600×.

FIG. 15. Conclusion of schizogony to produce trophozoites, showing the marked difference in the size of uninucleate cells thus produce. 1600×.

FIG. 16. Transverse fission of a 5-nucleate growth stage developed from a sporozoite, and indicating the irregularity of schizogony to produce trophozoites. 1600×.

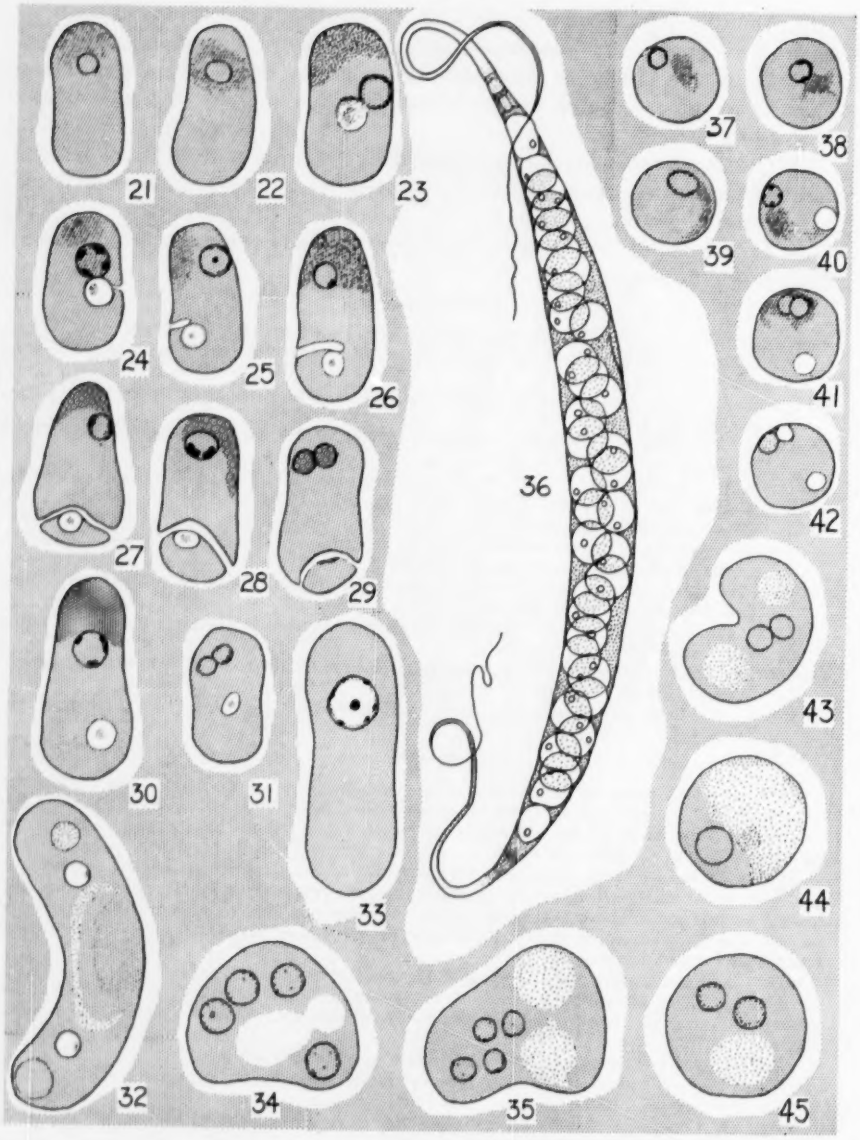
FIG. 17. Uninucleate products of the above division. 1600×.

FIG. 18. Early pre-gametocyst growth stage.

FIG. 19. A developing gametocyst having 8 nuclei. 1300×.

FIG. 20. A mature gametocyst containing 16 gametocytes in most of which polar nuclei are present. 1300×.

PLATE II



Amphiacantha ovalis (Figs. 21–35); *A. attenuata* (Figs. 36–45)

FIG. 21. Early gametocyte, showing the characteristic concentration of stainable cytoplasmic material at the end of the cell occupied by the nucleus.

FIG. 22. Gametocyte with oval shaped nucleus such as occurs just prior to division.

GANAPATHI, P. N. AND AIYAR, R. G. 1937 Life-history of a dicystid gregarine, *Lecudina brasili* n. sp., parasitic in the gut of *Lumbriconereis* sp. Arch. Protistenk. **89:** 113–132.

HARTMAN, O. 1944 Polychaetous annelids. Part V, Eunicea. Allan Hancock Pac. Exp. **10:** 1–236 (Univ. of So. Calif. Press, Los Angeles).

KAMM, M. W. 1922 Studies on gregarines II. Synopsis of the polycystid gregarines of the world, excluding those from the Myriapoda, Orthoptera, and Coleoptera. Illinois Biol. Monogr. **7:** 1–104.

KUDO, R. R. 1946 Protozoology, Ed. 3, 537 pp. (Thomas, Springfield, Ill.)

MACKINNON, D. L. AND RAY, H. N. 1931 Observations on dicystid gregarines from marine worms. Quart. J. Micr. Sc., Lond. **74:** 439–466.

NAVILLE, A. 1931 Les sporozoaires. Cycles chromosomiques et sexualité. Mém. Soc. Phys. et Hist. Nat. de Genève. **41:** (1), 223 pp.

REICHENOW, E. 1931 Sporozoa. In Die Tierwelt Nord- und Ostsee (Grimpe u. Wagler). Lief. 21, Teil I. d2. IIg, pp. IIgl–IIg, 88 pp. (Leipsig).

SHERESHEVSKAIA, E. G. 1924 La famille *Metchnikovellidae* (C. and M.) et la plaie (sic) qu'elle occupé dans le système des protistes. Russk. Arkh. Protist. **3:** 137–144.

FIG. 23. Gametocyte, after the first nuclear division, showing early degenerative changes in the polar nucleus.

FIGS. 24–26. Early stages in the formation of the cleft which will separate the polar cell.

FIGS. 27–28. Later stage, after complete separation of the polar cell.

FIG. 29. Conclusion of the second division of the functional nucleus. Note the last vestige of the first polar nucleus represented by a clump of stainable material in the polar cell adjacent to the cleft.

FIG. 30. Preparation of the functional nucleus for a second division, before the appearance of a cleft to separate the first polar nucleus in a pole cell.

FIG. 31. Conclusion of the second division of the functional nucleus, and a late stage in the degeneration of the first polar nucleus in a gametocyte in which no cleft is visible.

FIG. 32. Fusion of isogametes to form the prozygote. Note the vestiges of polar nuclei in the ends of the prozygote, and a central mass of stainable cytoplasmic material.

FIG. 33. Zygote with the synkaryon.

FIGS. 34–35. Four nucleate sporonts.

Amphiacantha attenuata (Figs. 36–45)

FIG. 36. Mature gametocyst containing 35 gametocytes prior to the beginning of maturation. 1300×.

FIG. 37. A young gametocyte prior to nuclear division.

FIGS. 38–39. Gametocytes in early stages of first nuclear division.

FIG. 40. Gametocyte, after first nuclear division showing the degenerating first polar nucleus across the cell from the functional nucleus.

FIG. 41. Isogamete at conclusion of the second nuclear division and prior to the disappearance of the first polar nucleus.

FIG. 42. Somewhat later, showing the beginning of degenerative changes in the second polar nucleus while still situated beside the functional nucleus.

FIG. 43. Fusion of isogametes to form the prozygote.

FIG. 44. Zygote with the synkaryon.

FIG. 45. A binucleate sporont.

NOTES ON THE MORPHOLOGY AND HOST-PARASITE SPECIFICITY OF *FIBRICOLA CRATERA* (BARKER AND NOLL, 1915) DUBOIS 1932 (TREMATODA: DIPLOSTOMATIDAE)

MARTIN J. ULMER
Iowa State College and Iowa Lakeside Laboratory

Adult frogs (*Rana pipiens*), collected from roadside ponds near the Iowa Lakeside Laboratory at Lake Okoboji in northwest Iowa, are heavily parasitized by strigeid diplostomula encysted in the pelvic and pectoral musculature. Similar infections have been found in frogs from farm ponds near Ames, Iowa. When such metacercariae were fed to laboratory-reared white mice, adult trematodes of the genus *Fibricola* (family DIPLOSTOMATIDAE) were recovered from the duodenum.

Reference to the literature indicates three species of this genus which have been recorded from the United States, namely: 1) *Fibricola cratera* (Barker and Noll, 1915) Dubois, 1932 [syns.: *Hemistomum craterum* Barker and Noll, 1915, *Fibricola laruei* Miller, 1940, *Fibricola nana* Chandler and Rausch, 1946]; 2) *Fibricola lucida* (LaRue and Bosma, 1927) Dubois and Rausch, 1950 [syns.: *Neodiplostomum lucidum* LaRue and Bosma, 1927, *Theriodiplostomum lucidum* (LaRue and Bosma) Dubois, 1944]; and 3) *Fibricola texensis* Chandler, 1942 [syn.: *Theriodiplostomum texensis* (Chandler) Dubois, 1944]. Additional species of the genus include *Fibricola caballeroi* Cerecero, 1943 from Mexico and *Fibricola minor* Dubois, 1936, found in Australia.

Of the three American species, which in nature parasitize a variety of mammals (muskrat, weasel, raccoon, mink, skunk, red squirrel, shrew, meadowmouse, and Norway rat), life-cycle studies relating to two have been published. The cycle of *Fibricola texensis* was demonstrated by Chandler (1942), and Cuckler (1940, 1949) reported briefly on that of *cratera*. *Fibricola lucida* is distinguished from both *cratera* and *texensis* by the presence in the adult worm of a well-marked constriction between the fore- and hind-body, and by the larger size of the adult. Less apparent differences distinguish adults of *cratera* and *texensis*. According to a taxonomic key to members of the genus presented by Dubois (1953), based in part upon those life-cycle studies noted above, the major distinction between the two species lies in the distribution of the vitellaria. In *texensis*, they extend from the bifurcation of the intestine to the level of the posterior testis and have a tendency to form two lateral bands in the hind-body. In *cratera*, the vitellaria extend from the bifurcation of the intestine to the level of the anterior testis or vitelline reservoir. Differences between the two species with reference to the intermediate stages include cercariae and metacercariae of smaller size in *texensis*. Moreover, metacercariae of *texensis*, according to Chandler (1942), occur as nonencysted diplostomula within the body cavity of tadpoles, while the displostomula of *cratera* migrate from the coelom and ultimately encyst in the pelvic musculature of the frog.

Several preliminary experiments with laboratory-reared white mice were conducted in the summer and fall of 1954. These involved the feeding of encysted

Received for publication, March 7, 1955.

metacercariae from pelvic muscles of *Rana pipiens* collected at Lake Okoboji. Periodic examination of the intestinal tract at varying times (see Table I) fol-

TABLE I.—*Results of feeding experiments*

Source of metacercariae	No. metacercariae fed	Hosts used	No. worms recovered	No. days between feeding and autopsy	Sexual maturity of worms
Pelvic muscles of *Rana pipiens*	?	white mouse	176	9	23 mature 153 immature
	several hundred	" "	59	15	all immature
	100	" "	7	18	all mature
	100	" "	76	20	all mature
	100	" "	83	26	74 mature 9 immature
	?	" "	118	27	all mature
	?	" "	11	28	2 mature 9 immature
	?	" "	7	29	all immature
	?	" "	2	59	1 mature 1 immature
	?	" "	7	70	all mature
	?	white rat	43	38	all immature
	?	" "	178	71	all immature
	50	1 day old chick	20	9	all mature
	50	10 day chick	0	8	
	50	10 day chick	0	9	
Intestine of *Rana pipiens*	100	white mouse	15	7	8 mature 7 immature

lowing such feedings led to the recovery of a series of worms, many of which were morphologically intermediate between *cratera* and *texensis*. The distribution of vitellaria was of particular interest, for in some specimens the follicles extended into the hind-body as far as the anterior level of the posterior testis (Fig. 1), while other adults exhibited vitellaria on one side of the body extending to the posterior end of the posterior testis (Fig. 12). Read (1948) noted that the only difference between *cratera* and *texensis* was that in the latter a thin sheet of vitellaria is limited to the ventral side of the hind-body. On the basis of this character, some specimens in the present collection could be considered as *texensis*.

Until very recently, the two species were separated from on another, too, on the basis of the condition of the diplostomula in the second intermediate host. Insofar as my specimens are concerned, since metacercariae were encapsulated in the pelvic muscles, they could be considered as *cratera*. Leigh (1954), however, reported that a species closely resembling *Fibricola texensis* in Florida produces encapsulated metacercariae and suggested that encapsulation may require more than the two months involved in Chandler's (1942) report dealing with the life cycle of *F. texensis*.

As indicated in the table, a number of adult worms were recovered from white mice which had been fed metacercariae found in the intestine of *Rana pipiens*. Such unencysted diplostomula were occasionally seen within the intestines of frogs from the same region near Lake Okoboji. The sexually mature worms recovered are indistinguishable from those obtained by feeding encysted metacercariae from muscles. Neither Chandler nor Cuckler in their experiments employed metacercariae obtained from the digestive tract of frogs. One possible explanation for the occurrence of the metacercariae in such an abnormal location in the second intermediate host is that older frogs may have eaten smaller, recently metamorphosed, infected frogs.

Measurements of unflattened, stained, and encysted diplostomula in teased frog muscle, from the same material in my feeding experiments show similarities to both *cratera* and *texensis*. On the basis of body length and width, my specimens resemble diplostomula of *texensis*, while measurements of oral sucker, acetabulum and holdfast are within the ranges indicated by Cuckler for *cratera*. Measurements of length and width of formalin-fixed, unflattened adult worms vary greatly in accordance with the degree of contraction of the specimens at the time of fixation and consequently appear to be of lesser diagnostic significance. Inasmuch as my experiments to date have dealt only with naturally-infected frogs, the possibility of double infections in a single frog must be considered. That the differences between *texensis* and *cratera* are exceedingly small as Read (1948) indicated, was confirmed by Dubois and Rausch (1950, pp. 43–44) who stated that the worms might perhaps represent only two physiological species.

It becomes apparent from the foregoing that the specimens here dealt with show affinities both to *Fibricola cratera* and *F. texensis*. Whether the two species are distinct will depend upon more detailed studies on the life cycles of these worms from the Okoboji region and elsewhere. Such studies, now in progress, should clarify the relationship between the American members of the genus.

Reproductive System. The relationships of the male and female organs conform generally to that previously described for the genus by Dubois and Rausch (1950). Since published reports dealing with American members of the genus do not include detailed morphological descriptions of the reproductive system, the following notes and illustrations (Figs. 1–12) are included in this report. Sections illustrated are from adults reared in white mice, fixed in Bouin's, cut at 10 microns and stained with Harris's and Mayer's haematoxylins, with erythrosin and eosin counterstains. Whole mounts, fixed in Bouin's or A.F.A., were stained with Mayer's alcoholic cochineal, Mayer's HCl carmine, Semichon's aceto-carmine and Harris's haematoxylin.

The ovary (Fig. 11) is situated laterally, tangential to the anterior testis, and near the junction of the fore- and hind-body. Posteriorly it gives rise to a short oviduct that expands into a small chamber which serves as a seminal receptacle from which a Laurer's canal extends dorsally, opening on the dorsal surface just posterior to the ovary. After giving off Laurer's canal, the oviduct turns ventrally, receives the vitelline reservoir (intertesticular in position) and, crossing to the opposite side of the body, forms a loop, then turns dorsally and enlarges somewhat to form the spindle-shaped ootype, surrounded by Mehlis' gland. Following a ventral and anterior course, the canal (now termed the ascending limb of the uterus) reaches the anterior level of the ovary and turning posteriorly, continues directly caudad as the descending limb of the uterus to the posterior of the hind-body. The uterus empties into a shallow dorsal, subterminal genital atrium.

The testes (Figs. 10, 12) are unequal in size and shape, the anterior one smaller, rounded or triangular; the posterior much larger, extending transversely across the body and exhibiting a median constriction. The small vasa efferentia arising from the testes unite medially near the front of the anterior testis, just behind the ovary, and form a rather large swollen seminal reservoir. The vas deferens originating at this point runs caudad just dorsal to, and parallel with, the descending limb of the uterus. At the posterior end of the hind testis it turns dorsally, expand-

ing gradually into a seminal vesicle which extends anteriorly approximately to the middle of the hind testis. Then turning back once more, the vas deferens continues posteriorly, narrowing gradually to form a weakly muscularized ejaculatory duct which joins the uterus shortly before the latter empties into the genital atrium.

Attachment of Adult. The attachment of the adult worms to the villi of the duodenum is tenacious. Serial sections of adults from white mice indicate that both oral sucker and acetabulum as well as the fore-body are involved (Fig. 13). The fore-body, whose sides are rolled ventrally, may completely encircle a villus (Fig. 14) and the holdfast is thus brought into intimate contact with the host's epithelial tissue. A dual mode of nutrition is apparently involved, through ingestion of host tissue by way of the oral sucker and digestive tract, and by diffusion through the tissue made possible by the close relationship between holdfast and the host's mucosa. Although sections show some evidence of tissue erosion where the holdfast is in intimate contact with host tissue, erosion is apparently far less than that reported among other larger strigeids where holdfast and the inner surface of the fore-body function as nutritive structures.

Host-parasite Specificity. In a recent supplement to his monograph on the strigeids, Dubois (1953) continues to employ host-specificity as a major taxonomic criterion for the separation of the two subfamilies of the DIPLOSTOMATIDAE. Thus, members of the DIPLOSTOMATINAE are considered as parasites of birds, while the ALARIINAE (to which *Fibricola* is assigned) parasitize mammals exclusively. In view of recent experiments noted below, such a separation on the basis of definitive host involved seems open to question.

Encysted metacercariae of *Fibricola* from the same frogs employed in rearing the adults noted above were fed to laboratory-raised chicks which varied in age from one to ten days. As shown in the table, of 50 metacercariae fed to a one-day old chick, 20 sexually mature worms were recovered 9 days post-feeding. Experiments involving chicks older than one day at the time of feeding gave negative results. This confirms Leigh's (1954) findings that day-old chicks serve as suitable definitive hosts for *Fibricola*. He noted, however, that by 10 days most worms are eliminated by chickens. Inasmuch as both mammalian and avian hosts, then, are apparently capable of harboring adults of *Fibricola*, the following statement of Dubois and Rausch (1950: 45) appears questionable and probably will require modification:

". . . Nous pensons que le genre *Fibricola* Dubois devra être défini à la fois par le caractère anatomique exprimant la tendance au confinement des follicules vitellogènes dans le segment antérieur, et par le caractère biologique fondé sur l'adaptation de ses especes à la classe des Mammifères."

Whether avian hosts in nature harbor members of the genus *Fibricola* remains to be seen. Studies now under way should answer this as well as other questions regarding this extremely interesting group of helminths.

LITERATURE CITED

CHANDLER, A. C. 1942 The morphology and life cycle of a new strigeid, *Fibricola texensis*, parasitic in raccoons. Tr. Am. Micr. Soc. **61**: 156–167.

CUCKLER, A. C. 1940 The life cycle of *Fibricola cratera* (Barker and Noll, 1915) Dubois, 1932 (Trematoda: Strigeata). J. Parsit. **26** (Suppl.): 32–33.

———1949 Morphological and biological studies on certain strigeid trematodes of mammals. Summaries Ph.D. Theses, U. Minnesota, IV: 45–47.

DUBOIS, G. AND RAUSCH, R. 1950 Troisième contribution à l'étude des *Strigeides (Trematoda)* nord-américains. Bull. Soc. Neuch. Sc. nat. **73:** 19–50.

DUBOIS, G. 1953 Systématique des Strigeida. Complément de la Monographie. Mem. Soc. Neuch. Sc. nat. **8:** 1–141.

LEIGH, W. H. 1954 Notes on the life history of *Fibricola texensis* Chandler, 1942, in Florida. J. Parasit. **40** (No. 5–Sect. 2): 45.

READ, C. P. 1948 Strigeids from Texas mink with notes on the genus *Fibricola* Dubois. Tr. Am. Micr. Soc. **67:** 165–168.

EXPLANATION OF PLATES

(All drawings were made with the aid of the camera lucida.)

FIG. 1. Partial reconstruction of female reproductive system from sagittal sections.

FIGS. 2 through 9. Representative cross-sections through adult. All drawn to same scale.

FIG. 2. Cross-section through junction of ejaculatory duct and uterus.

FIG. 3. Cross-section through posterior region of hind testis.

FIG. 4. Cross-section through mid-region of posterior testis.

FIG. 5. Cross-section through region of vitelline reservoir.

FIG. 6. Cross-section through region of oviduct, seminal receptacle and Laurer's canal.

FIG. 7. Cross-section through ovary.

FIG. 8. Cross-section through holdfast.

FIG. 9. Cross-section through acetabulum.

FIG. 10. Reconstruction of male reproductive system from frontal sections.

FIG. 11. Reconstruction of female reproductive complex from cross-sections.

FIG. 12. 20-day adult from white mouse, ventral view.

FIGS. 13, 14. Adult worms *in situ*. 70-day adults from duodenum of white mouse. Serial sections showing attachment of adult to villus by means of oral sucker, acetabulum and forebody.

ABBREVIATIONS USED

A—acetabulum
EB—excretory bladder
ED—ejaculatory duct
EP—excretory pore
GA—genital atrium
H—holdfast
HG—glands of holdfast
IC—intestinal caecum
LC—Laurer's canal
MG—Mehlis' gland
O—ovary
OS—oral sucker
OVD—oviduct
P—pharynx
SR—chamber serving as seminal receptacle
SRV—seminal reservoir
SV—seminal vesicle
T_1—anterior testis
T_2—posterior testis
UTA—ascending limb of uterus
UTD—descending limb of uterus
VD—vas deferens
VE_1—vas efferens of T_1
VE_2—vas efferens of T_2
VF—vitelline follicle
VR—vitelline reservoir
VTD—vitelline duct

PLATE I

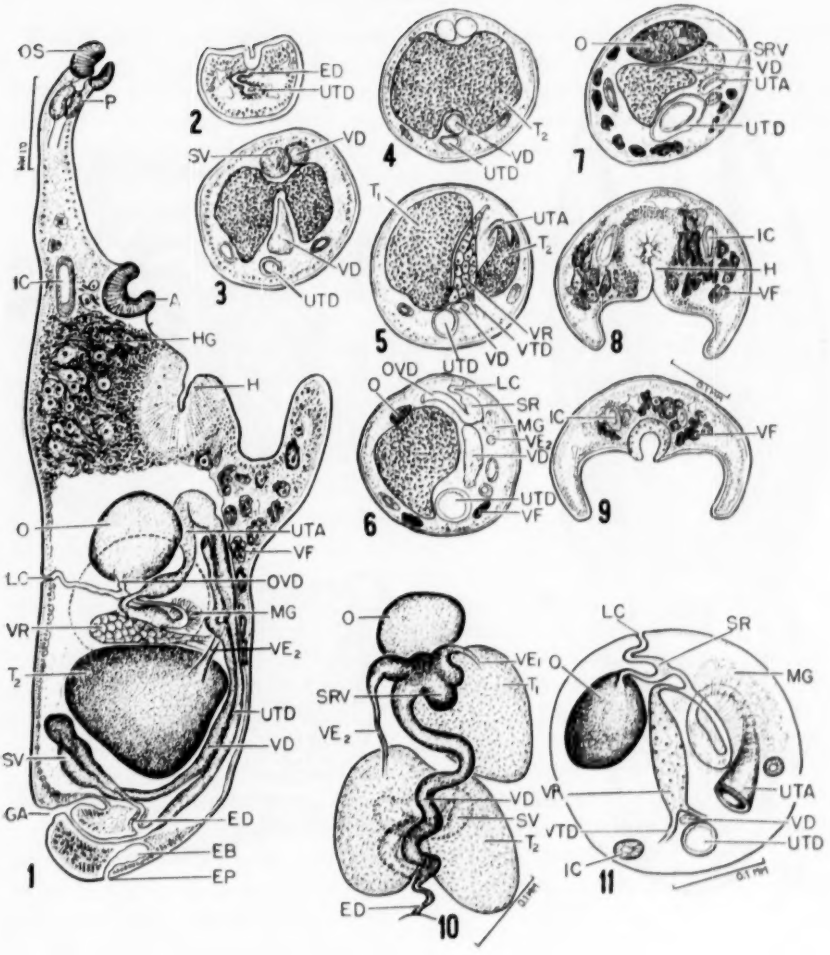
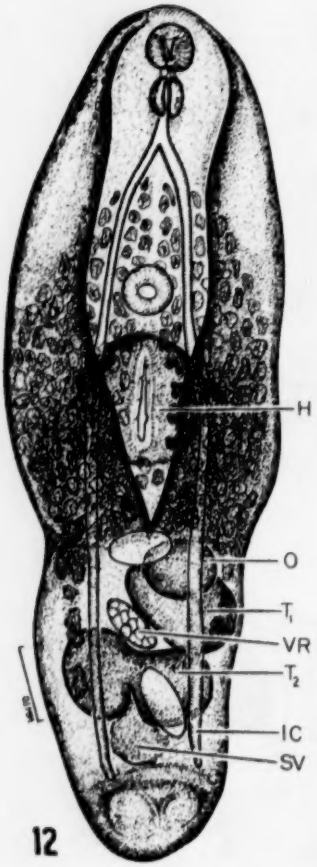
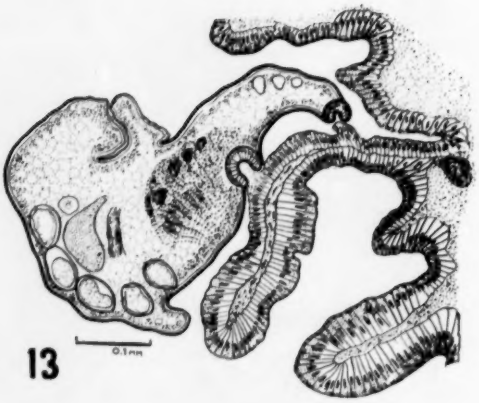

OS
P
IC
A
HG
H
O
UTA
VF
LC
OVD
VR
MG
VE2
T2
UTD
VD
SV
GA
ED
EB
EP
1
ED
UTD
2
SV
VD
VD
UTD
3
T2
VD
UTD
4
T1
UTA
T2
VR
VTD
VD
5
OVD
UTD
LC
O
SR
MG
VE2
VD
UTD
VF
6
O
SRV
VD
UTA
UTD
7
IC
H
VF
8
IC
VF
9
O
VE1
T1
SRV
VE2
VD
SV
T2
ED
10
LC
SR
O
MG
VR
UTA
VTD
VD
UTD
IC
11

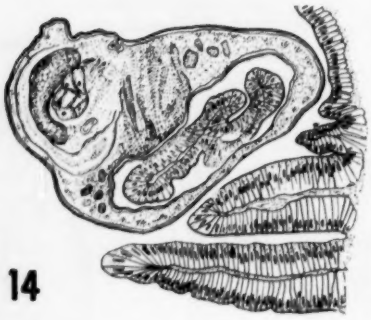
PLATE II



12



13



14

SUSCEPTIBILITY STUDIES IN SCHISTOSOMIASIS. IV. SUSCEPTIBILITY OF WILD MAMMALS TO INFECTION BY *SCHISTOSOMA HAEMATOBIUM* IN EGYPT, WITH EMPHASIS ON RODENTS[1]

ROBERT E. KUNTZ[2] AND GEORGE M. MALAKATIS[3]
U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

In recent years a number of reports have discussed the susceptibility of various mammals to infection by the schistosomes of man and domestic animals (Kuntz, 1955). *S. mansoni* has assumed the position of greatest importance in this field of study since it has been available to many laboratories and can be reared rather easily in artificial environments. Experimental studies in schistosomiasis haematobia, on the contrary, have been somewhat limited since this parasite and its snail host are not well adapted to laboratory culture.

Investigation into the different phases of experimental schistosomiasis at Cairo has permitted us to undertake a series of susceptibility studies relating to both *S. mansoni* and *S. haematobium*. The present study, the fourth in a series (Kuntz, *et al.*, 1954, 1955a, 1955b), was made in conjunction with other research on the biology of the schistosomes. Data, as presented in this paper, may be easily compared with similar information obtained from the infection and examination of both laboratory and wild mammals for schistosomes in previous studies.

MATERIALS AND METHODS

Representatives of most of the smaller species of mammals common to Lower Egypt, and a few larger animals, were exposed to cercariae of *S. haematobium*, employing when feasible, laboratory raised hosts just approaching maturity. Approximately half of the *Mus, Rattus, Arvicanthis, Acomys* and *Meriones* used were obtained as offspring of adults which had reproduced in captivity. The remainder were taken from litters of young near the weaning age. These accompanied the mothers brought in from the wild. Half grown desert rodents (*Jaculus, Gerbillus* and *Psammomys*), bandicoots, hedgehogs and young mongooses and foxes were selected from stocks provided by native collectors. Weasels were trapped in homes and in public buildings in Cairo.

Procedures for infection, handling, examination and tabulation of data were essentially the same as described in previous reports in this series. Briefly, all hosts were given a pre-exposure immersion in warm water to encourage the passage of urine and feces; the smaller hosts were exposed to 200 cercariae in half an inch of water in appropriate size glass containers; and the larger mammals, the mongooses and foxes, were exposed to estimated numbers of cercariae, i.e. 1500 to 2000 cer-

Received for publication, March 23, 1955.

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

[2,3] Present address: Parasitology Departments of the Naval Medical School and the Naval Medical Research Institute respectively, NNMC, Bethesda, Md.

The authors are indebted to Harry Hoogstraal, Head of Department of Medical Zoology, Naval Medical Research Unit No. 3, who gave assistance in obtaining the wild rodents used. He also has provided the authors with host identifications.

cariae and 4000 to 5000 cercariae respectively. All hosts remained in contact with the cercarial suspension for 30 to 45 minutes. Cercariae of *S. haematobium* were pooled from the daily natural emergence from 20 to 30 infected *Bulinus truncatus* dipped from irrigation systems near Cairo.

Examination of hosts was made at death or at different intervals after the schistosomes had reached maturity. As in previous studies a number of hosts were allowed to live for longer periods to determine whether duration of infection would influence the recovery of schistosomes, location of parasites in the host, degree and sites of egg deposition, etc. Hosts were killed with chloroform. The abdominal and thoracic organs were removed to allow examination of each separately with the aid of a dissecting microscope. Parasites were removed as the organs were macerated with splinter forceps, then the tissues were re-examined for parasites after thorough washing in physiologic saline.

Measurements were taken from intact worms after standing in physiologic saline solution for several hours. Enlargement of spleens in infected animals was judged by comparison with the size of spleens removed from noninfected control hosts.

RESULTS AND DISCUSSION

A total of 278 mammals was exposed to infection: 256 rodents (represented by nine species); 14 hedgehogs; and 8 carnivores (3 weasels, 2 mongooses and 3 foxes). Pertinent findings are indicated in Table 1. Data obtained from a study of the larger hosts, i.e. the carnivores, are not included in this table since the circumstances relating to their exposure were quite different.

The majority of the smaller species of mammals harbored schistosomes when examined or showed evidence of infection some time prior to autopsy. Some of the individuals of each host species of the rodents and insectivores were infected. However, there was considerable range in the manifestation of parasitism and in the recovery of schistosomes. Gross pathology covered a wide range of involvement, the extent of damage to various organs depending upon the fecundity and location of the parasites, as well as upon the individual host's reaction to the presence of eggs.

Mus musculus praetextus Brants, the house mouse.—Although the overall recovery of schistosomes from house mice was lower than that recorded previously for albino mice (Kuntz and Malakatis, 1955b), all but one of 33 house mice were infected when examined. The yield of parasites from the few hosts which lived for one year or longer was approximately equal to that recorded from hosts examined after much shorter periods of infection. The majority of parasites in the three age categories was found in the intrahepatic and portal vessels near the liver. Schistosomes in the mesenteric system were located posteriorly in the inferior branches of the mesenteric vein. A few small worms were found in the lungs of mice of the oldest age group but none was present in other organs.

An examination of mated female schistosomes indicated that production of normal fertile eggs began during the 10th or 11th week. At the time up to 20 immature or nonfertile eggs were also observed in the uterus of some parasites and a few nonviable eggs had been deposited in nearby tissues. By the end of 14 weeks numerous viable eggs were observed in tissues, as well as in the uterus of worms. Apparently, egg production reached its height at 15 to 17 weeks, since some females possessed as many as 91 eggs. The intervals at which eggs were passed during this period

TABLE 1.—*Parasites recovered, and distribution of parasites and their eggs in wild mammals exposed to 200 cercariae of* Schistosoma haematobium

Hosts	Duration of infection (weeks)	Number of hosts examined	Deaths among hosts exposed (%)	Parasites recovered at autopsy		Sex of parasites (%)		Parasite measurements (mm.)				Location of parasites in hosts					Hosts (percentage) with eggs in tissues of:					Hosts (%) showing splenomegaly
								Males		Females		Total No. (%) of parasites in veins of:										
				Range	Percentage	Males	Females	Range	Mean	Range	Mean	Liver*	Small and large intest.**	Lungs	Spleen	Bladder	Liver	Small intest.	Large intest.	Lungs	Spleen	Bladder	
Rodents:																							
Mus musculus	8–14	14	0	0–27	4.7	70	30	7.7–15.4	12.4	10.1–19.1	15.2	59	41	0	0	0	71	0	36	0	0	0	28
	16–22	15	13	2–18	4.0	51	49	9.0–15.5	12.9	12.0–19.0	15.6	70	29	1	0	0	73	29	67	0	7	7	40
	50–68	4	50	4–19	4.0	62	28	11.0–16.5	14.4	18.0–21.5	19.5	61	29	10	0	0	100	25	75	25	25	0	25
Rattus rattus	11–18	17	6	0–12	0.8	64	36	8.0–11.0	8.9	5.0–11.5	9.0	61	39	0	0	0	18	6	0	0	0	0	0
Arvicanthis niloticus	7–11	10	10	22–48	17.8	56	44	8.4–13.3	11.6	11.1–17.8	15.2	59	41	0	0	0	70	20	50	0	0	0	0
	16–22	16	0	1–50	14.6	61	39	9.4–17.0	14.0	13.7–20.4	17.4	56	42	0	0	12	87	7	87	12	6	19	31
	45–68	4	50	0*–13	4.5	48	52	13.0–17.1	14.2	14.3–20.5	17.3	52	48	0	0	0	100	25	75	25	25	25	50
	98–141	4	50	3–20	3.8	53	47	14.1–17.2	14.5	14.6–24.0	17.6	23	47	0	0	0	75	0	50	0	0	25	25
Acomys cahirinus	8–12	13	15	7–54	11.2	55	45	6.1–12.6	11.3	8.2–19.4	14.6	52	48	0	0	0	69	8	78	0	0	0	8
	16–22	17	18	0*–30	4.6	49	51	8.8–14.5	11.8	12.3–17.1	14.7	71	29	0	0	0	76	6	59	0	0	0	12
	30–43	5	20	3–13	3.9	38	62	—	—	—	—	87	13	0	0	0	100	20	100	0	0	0	0
Gerbillus pyramidum	7–12	21	10	5–40	10.0	53	47	5.9–13.1	11.1	8.1–18.6	14.2	51	49	0	0	0	71	19	71	0	0	0	5
	16–22	18	6	3–50	10.2	50	50	10.6–14.3	13.4	11.8–19.1	17.7	42	57	0	1	0	100	72	89	6	6	0	22
	64–113	5	40	0	0	—	—	—	—	—	—	0	0	0	0	0	80	60	60	20	0	20	20
Jaculus jaculus	11–17	30	7	0–8	0.6	67	33	5.8–12.3	9.7	12.6–14.6	13.5	73	27	0	0	0	10	0	13	0	0	0	0
Meriones shawi	11–18	16	13	0–21	2.8	70	30	2.1–14.1	10.0	13.5–18.2	15.7	90	8	2	0	0	19	7	25	7	0	13	25
	23–75	13	15	0–11	1.6	54	46	9.3–17.0	12.0	14.6–22.6	19.1	80	20	0	0	0	46	38	46	8	8	23	8
Psammomys obesus	11–17	11	18	4–22	5.7	67	33	9.7–16.0	12.2	13.7–19.8	16.9	50	50	0	0	0	82	18	64	9	0	0	0
Nesokia indica	6–12	8	75	8–36	11.5	57	43	7.3–13.0	11.6	9.5–18.9	15.9	39	61	0	0	0	13	63	13	0	0	0	13
	16–30	11	73	1–38	10.4	48	52	9.0–16.5	13.5	12.8–23.2	18.1	56	43	1	0	0	82	82	18	9	9	18	18
	40–55	4	100	5–78	14.7	54	46	11.0–16.5	14.0	17.3–24.2	19.1	36	64	0	0	0	100	75	50	25	25	25	25
Insectivores:																							
Hemiechinus auritus	14–28	12	33	0–78	7.7	50	50	10.8–14.8	12.6	11.7–24.0	18.4	84	26	0	0	0	75	58	42	0	0	0	17
	58–62	2	100	0†	0	—	—	—	—	—	—	0†	0	0	0	0	50	100	50	0	0	0	0

* Liver and hepatic-portal system.
** Mesenteric veins of small and large intestine.
† Hosts, though negative for parasites, showed definite evidence of infection at earlier date.

were irregular and the quantity was variable. Fewer eggs were observed in schistosomes of hosts examined two years after infection and numbers of eggs in tissues near the parasites were limited.

Egg deposits were more extensive in the large intestine and associated mesenteries than in other organs, although the majority of hosts possessed eggs in the tissues of the liver. Most livers were normal in appearance, there was little pigmentation and only a few deposits of eggs were apparent macroscopically on the surface of this organ. Pathologic damage was not as severe as observed earlier in house mice infected with a similar load of *S. mansoni* (Kuntz and Malakatis, 1955a) and cirrhosis, even in the hosts with moderate deposits of eggs, was not great. The majority of eggs were distributed throughout the deeper tissues of the liver. Most were non-viable, collapsed and in different stages of destruction. Eggs in groups ranging from 20 to 150 and even to 200 were deposited as clumps in and on the intestinal wall. Deposits of viable eggs were frequently found near an egg-producing female schistosome. In some instances numerous eggs beneath the outer covering of the intestine formed macroscopic protuberances encircling the intestine. Two hosts had obvious macroscopic ulceration of the walls of the large intestine. Hemorrhage was prominent and many eggs were found in the surrounding tissues upon microscopic examination. One of these two hosts apparently died as a result of loss of blood. Viable eggs were present in the intestinal contents of both mice. A few eggs, but no worms, were found in the spleen and in the region of the urinary bladder. On no occasion were eggs recovered from the urine.

A greater percentage of house mice showed splenomegaly than other hosts employed. However, there was no correlation between this condition and presence of parasites and their deposits of eggs.

Rattus rattus (L.), the common house rat.—House rats have been classed as poor hosts for the schistosomes of man, although the recovery was greater than for albino rats (Kuntz and Malakatis, 1955b), exposed to an equal number of cercariae. Only 6 of 17 hosts had parasites. These were removed from the vessels draining the lower small intestine, as well as from the intrahepatic veins of the liver. Female schistosomes removed from both sites, though stunted, possessed, a few viable eggs in the uterus. The few eggs present in the liver were widely scattered, non-viable and had provoked no marked tissue response other than fibrotic enclosure of the individual eggs. Male schistosomes were in the majority and there was no evidence of splenomegaly.

Arvicanthis niloticus (Desmarest), the Nile Kusu or Nile rat.—From the standpoint of general susceptibility, size of host and development of seemingly normal egg-laying schistosomes, the Nile rat was considered the most ideal host of the species represented. Viable eggs were passed in the feces of this host for approximately three years. However, as indicated in previous work employing small animals, even in those considered as acceptable hosts there was a definite tendency toward reduction in numbers of parasites in prolonged infections.

All *Arvicanthis* possessed schistosomes or other evidence of infection. Eggs first appeared in the liver by the end of eight weeks. Deposits in the mesentery and walls of the large intestine were observed during the 9th week, then two weeks later a few eggs were found in the walls of the small intestine. By the 12th to 13th

week there were numerous eggs, many viable, in the liver of some hosts. Later the proportion of non-viable eggs became greater as there was evidence of formation of small fibrotic patches in the liver tissue. Pigment formation was only moderate at its greatest and the liver never attained the state of sclerosis so often witnessed in Nile rats infected with a similar number of adult *S. mansoni*. At the height of egg production some hosts had hundreds of eggs in the wall of the cecum and other parts of the large intestine but there were only limited numbers in the small intestine. Hemorrhagic lesions were present in some hosts and most passed eggs with feces or eggs were present in the intestinal contents at autopsy. Egg deposits in some instances extended from large aggregates on the intestinal wall into the adjoining mesenteries and fat bodies but tissue damage or reaction seemed to be less than would be expected considering the abundance of eggs.

One pair of worms was removed from a vessel near the urinary bladder and three of the rats in the second age group harbored moderate numbers of eggs in the tissues at the base of the bladder. Scattered strings or nests of eggs occurred in the bladder wall proper. At autopsy bladder lesions and blood were obvious macroscopically and viable eggs were obtained from the urine of the bladder. Each of these hosts had passed moderate quantities of eggs detected in naturally passed urine. Eggs but no schistosomes were associated with the urogenital system of hosts in the older age groups. As indicated in Table 1, eggs were found also in other organs.

Counts of eggs *in utero* revealed interesting facts. The first mature eggs appeared in the female schistosomes at 8 to 9 weeks after date of infection. There was a notable increase in number of eggs, i.e., 3 to 14 at 9 weeks, to a maximum of 88 eggs at 16 to 22 weeks. During the latter period the average was 36 eggs per parasite. Apparently there was fluctuation in egg production as indicated by *in utero* counts and by passage of eggs in fecal pellets. Several well developed females in the period usually considered as representing the height of the egg-laying cycle, i.e. 16 to 22 weeks, showed only 2 to 8 fertile eggs in the uterus. Two females more than two years of age and one with the unusual length of 24.8 mm. had no mature eggs in the uterus, although their ovaries were apparently functional.

Splenomegaly occurred in hosts in all but the first age group, but the degree of enlargement was less pronounced than in infected house mice. Hosts with thousands of eggs along the entire length of the large intestine, parts of the small intestine, around the bladder and with moderate numbers of eggs in the liver possessed spleens of normal size. As in previous studies with *Arvicanthis* infected with *Schistosoma mansoni*, it was possible to recover eggs from rectal scrapings of living hosts.

Acomys cahirinus (Desmarest), the spiny-back mouse.—The spiny-back mouse was readily susceptible to infection by *S. haematobium* and occupied a position equal to that of *Mus musculus*. All hosts harbored schistosomes or showed unquestionable evidence of earlier infection. The smaller body size of this rodent may have accounted for the presence of fewer worms and egg deposits in the lower part of the mesenteric circulation and for an absence of eggs in tissues around the urinary bladder. In general pathologic involvement of the liver and parts of the intestine paralleled that observed in *Mus*. However, none of the *Acomys* had macroscopic sites of hemorrhage or intestinal ulceration. Adult *S. haematobium* from the spiny-back mice were slightly smaller than those recorded from *Mus* and *Arvicanthis*.

In this respect, it should be stated that the number of eggs *in utero* for hosts in all age categories of *Acomys* was lower. Only limited numbers of eggs were passed with feces.

Gerbillus pyramidum Geoffrey, the gerbil or common desert mouse.—The pattern of parasitism in the gerbil was similar to that observed in *Mus* and *Acomys*. Five of 44 gerbils were negative when examined for schistosomes, but four of these had viable eggs in the intestine and liver. Egg deposits were not extensive and there was evidence of intestinal ulcerations with loss of blood in only one host. In this host the spleen was slightly enlarged and the body cavity was filled with fluid 21 weeks after infection.

A few worms reached the walls of the large intestine, but mated females had fewer eggs *in utero* than those from *Arvicanthis*. Restricted egg production by parasites and thus a minimum of tissue damage in the gerbils was reflected in the low death rate among animals exposed. In one host, infected for two years, no worms were found at autopsy, but there were egg deposits in the lungs and in the walls of the urinary bladder. This gerbil had passed viable eggs with feces 10 to 12 weeks prior to autopsy.

Jaculus jaculus L., the gerboa or desert jumping mouse.—From the standpoint of general susceptibility, the gerboa falls into the same category as *Rattus*, since the recovery of parasites was less than 1 percent and eggs were produced in only four hosts. Seventeen of the 30 gerboas when autopsied harbored no worms and in 15 of the 17 there was no evidence of parasite residence.

Counts of eggs *in utero* seemed to indicate better than average compatibility of parasite and host in two of the 30 hosts. In these, the females were larger than average and the uterus of one contained 24 and the other 58 viable eggs; while smaller mated females from other individuals in which the parasite recovery was low had no mature eggs in the uterus at the end of 16 weeks. Tissue damage in the gerboas was only slight.

Meriones s. shawi Duvernoy, the jird, a desert rodent.—The jird is classed as a rather poor host, if judgment is based solely on the recovery of adult *S. haematobium*. Seven of 29 hosts were negative for worms and only 2 of the 7 gave evidence of previous parasitism. *Meriones*, however, stood out from the other species since egg distribution within the body was more generalized. Eggs were deposited in the lungs, in the spleen, along the small and large intestines and in the walls of the bladder, in spite of the fact that the greater proportion of schistosomes were removed from the region of the liver. In two hosts there were numerous eggs around the prostate gland and there was blood in the bladder urine. Pathologic involvement of the intestine, however, was less marked than that recorded for other hosts. Splenomegaly, when present, was very pronounced in the hosts examined at the end of 15 to 18 weeks. Spleens were enlarged 2.5 to 3 times normal size.

Psammomys o. obesus Cretzschmar, the fat sand rat.—The percentage return of schistosomes from the fat sand rat was only moderate but the number of parasites in the individual hosts was more consistent than in other species of hosts employed. All hosts were infected. There were only moderate deposits of eggs even in the large intestine, although half of the schistosomes were found in branches of mesenteric veins associated with this organ system. Eggs when present were scattered throughout the liver but were too few to provoke marked tissue reaction. Many

of the eggs were partially destroyed or surrounded by small nodules of fibrous tissue. The host seemed to tolerate the few patches of eggs at different sites along the intestine without showing evidence of drastic tissue reaction and formation of open macroscopic lesions into the intestine. *In utero* egg counts were not high, i.e. 6 to 53. No eggs were deposited in the urogenital system.

Nesokia indica suilla Thomas, the Egyptian bandicoot.—There were moderate recoveries of schistosomes from bandicoots, the greatest number of worms being found in hosts examined approximately one year after infection. A greater number of schistosomes was found in both the superior and inferior branches of the mesenteric veins and they produced greater quantities of eggs than worms observed in other hosts. This, of course, resulted in more extensive damage to organs and a much higher death rate than recorded for the other host species. Masses of eggs encircled both the small and large intestine, forming macroscopic protuberances inside and outside the intestinal wall. Pigmentation was obvious in the intestinal wall and in the mesenteries. There were numerous lesions and sites of hemorrhage. In several instances, the intestinal contents included quantities of blood and mucus and viable eggs were present in considerable numbers. There were no worms, but moderate numbers of eggs were deposited in the walls of the urinary bladder and eggs were found in the urine. Eggs had been passed with the feces for several weeks before autopsy.

Several mated females, lying in vessels adjacent to the large intestine, had more than 100 eggs *in utero* at 18 to 22 weeks. One schistosome at 55 weeks of age had 108 eggs *in utero*, while another had only 18. The distribution of eggs was similar to that recorded for *Arvicanthis* and may well be an expression of more favorable host-parasite relationships due, in part, to the comparatively larger size of these hosts. Egg deposits were detected in the spleen and lungs, as well as along the greater length of the intestine. There was moderate cirrhosis in several livers with large deposits of eggs. Hepatomegaly as well as splenomegaly was obvious in two hosts. The bandicoot could be classed as an excellent host if one were interested primarily in the pathological aspects of experimental schistosomiasis with *S. haematobium*. This host, however, has an unpredictable disposition and did not reproduce in captivity.

Hemiechinus auritus aegyptius Fischer, the Egyptian hedgehog.—This insectivore is classed as a fair host for *S. haematobium*. Two of the 3 hedgehogs which possessed no worms at autopsy still had deposits of eggs in the intestine and in the liver. *In utero* counts of eggs were much lower than in the schistosomes from *Arvicanthis* or *Nesokia*, i.e. 0 to 48, with an average of 16, at 15 to 18 weeks after infection. Egg deposits in the different systems were limited. The majority were non-viable and calcified and there were no hemorrhagic lesions in the intestinal walls. One hedgehog with 12 pairs of schistosomess had only a very few eggs deposited in tissues but the spleen was approximately 4.5 times normal size.

Mustela nivalis subpalmata (Hemprich and Ehrenberg), the Egyptian weasel; *Herpestes i. ichneumon* L., the Egyptian mongoose; and *Vulpes v. aegyptiaca* Sonnini, the Egyptian fox.—All carnivores were negative and there was no evidence that infection had occurred. The weasels were exceedingly difficult to handle and the three exposed to cercariae lived no more than 8 to 11 days. Negative results were none too surprising since the carnivores had been shown in earlier studies (Kuntz,

et al., 1954, 1955a and 1955b) to be very poor or non-susceptible to infection by either *S. mansoni* or *S. haematobium*.

Judging from the information given above, it is obvious that, under conditions conducive to infection, *S. haematobium* is capable of establishing itself in a number of different hosts. Parasite and host compatibility varies considerably among the individuals of any one species, as well as from one host species to another. The welfare of *S. haematobium* in a given host may be judged, in part, on the basis of such criteria as the *in utero* egg counts in female parasites and the relative deposition of eggs in tissues of the host. Our observations indicate that tissue damage along the intestine may be quite localized and closely related to the presence of an egg-laying schistosome. Severity of tissue damage is usually dependent upon the fecundity of the parasite in a given location. Passage of viable eggs in feces and urine, it would seem, depends upon the location of egg-producing females in the host and upon the formation of lesions at favorable sites to permit escape of eggs. Limited numbers of eggs may be associated with the urogenital system or may reach the walls of the urinary bladder, in spite of the fact that adult *S. haematobium* reach this part of the circulation in small animals only on rare occasions. Only limited quantities of eggs were detected in naturally passed urine.

Reports upon the susceptibility of small mammals to infection with the schistosomes of man and related species are variable and in some cases contradictory. Although these studies have dealt with *S. mansoni*, it is now apparent that both *S. mansoni* and *S. haematobium* are capable of infecting a considerable number of species of laboratory and wild mammals. Rodents make up a prominent part of the hosts listed.

Reports of infection of small mammals other than laboratory hosts with *S. haematobium* are few. Leiper (1918), in attempting to solve the life cycle of the schistosomes of man in Egypt stated that desert rats had been infected but he failed to name the host species and give the results obtained. *Pachyuromys duprasi*, the fat-tailed sand jerboa of Egypt, was infected with *S. haematobium* in Dr. Barlow's laboratory in Cairo (Stunkard, 1946). Small non-viable eggs were passed with the feces of this host and parasites subnormal in size were removed from the mesenteric veins at autopsy following death. Gerbils (*Gerbillus*) exposed to infection with *S. haematobium* by Abdel Azim and Cowper (1950) gave a small yield of schistosomes. The same investigators exposed hedgehogs to *S. haematobium* in an attempt to repeat the earlier work of Brumpt (1928) who stated that both worms and eggs were found in these hosts. Abdel Azim and Cowper, however, failed to recover *S. haematobium* from hedgehogs, although a few terminal spined eggs were passed with feces of one host.

Susceptibility studies have contributed considerably to our understanding of different phases in the basic biology of the schistosomes and it is now apparent that the species found in man are not nearly so host specific as at one time supposed. It is unnecessary to expand on a discussion of the relationship that such studies hold in this field of parasitology, since the subject has been recently reviewed (Kuntz, 1955).

SUMMARY

A total of 278 mammals (9 species of rodents, an insectivore, and 3 species of carnivores) were exposed to cercariae of *Schistosoma haematobium* under condi-

tions conducive to infection. The results obtained indicate that the rodents as a group were the most susceptible to infection, whereas the carnivores were not susceptible. The hedgehog, an insectivore, was considered as only a fair host.

Arvicanthis niloticus was judged as the best all-round host since it provided a good return of seemingly normal *S. haematobium* which deposited numerous eggs in different organs. Viable eggs were passed with the feces for approximately three years, there was pathologic involvement of the urogenital system and a few viable eggs were passed with the urine.

In utero counts of eggs in the female schistosomes coupled with observations on distribution and extent of egg deposits in various tissues, in general, served as criteria for judging host-parasite relationships.

References Cited

Abdel Azim M., and Cowper, S. G. 1950 On the maintenance of strains of *Schistosoma mansoni*, *S. haematobium* and *S. matthei* in the laboratory in Egypt, with special reference to the use of gerbils. Brit. J. Exper. Path. **31**: 577–589.

Brumpt, E. 1928 Réalisation expérimentale du cycle complet du *Schistosoma haematobium*. Ann. Parasitol. **6**: 440–446.

Kuntz, R. E. 1955 Biology of the schistosome complexes. Am. J. Trop. Med. and Hyg. **4**: (In press).

——— and Malakatis, G. M. 1955a Susceptibility studies in schistosomes. II. Susceptibility of wild mammals to infection by *Schistosoma mansoni* in Egypt, with emphasis on rodents. Am. J. Trop. Med. and Hyg. **4**: 75–89.

——— and ———1955b Susceptibility studies in schistosomiasis. III. Infection of various experimental hosts with *Schistosoma haematobium* in Egypt. Exper. Parasitol. **4**: 1–20.

———, ———, and Wells, W. H. 1954 Susceptibility of laboratory animals to infection by the Egyptian "strain" of *Schistosoma mansoni*, with emphasis on the albino mouse. 5. Congr. Internat. Méd. Trop. et Paludisme, Istanbul. **2**: 374–391.

Leiper, R. T. 1918 Report on the results of the Bilharzia mission in Egypt, 1915 Part V. Adults and ova. J. Roy. Army Med. Corps, **30**: 235–260.

Stunkard, H. W. 1946 Possible snail hosts of human schistosomes in the United States. J. Parasitol. **32**: 539–552.

THE USE OF AN ACID SOLUBLE PROTEIN FRACTION IN THE FLOCCULATION TEST FOR THE DIAGNOSIS OF TRICHINOSIS

ELVIO H. SADUN AND L. NORMAN
Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia

Melcher (1943) isolated six fractions from lyophilyzed *Trichinella spiralis* larvae. Skin and precipitin tests in experimentally infected rabbits revealed that the acid soluble protein fraction reacted in high dilutions. He concluded that this "is probably the fraction responsible for the positive skin test and serological reactions described by previous investigators working with less refined extracts of the larvae."

On the other hand, working with humans, Frisch and his co-workers (1946) found that the acid soluble protein was distinctly less reactive than the crude extract of larvae. They suggested that the results "may merely indicate a species difference to immunization with larvae."

Bozicevich and his co-workers (1951) developed a practical and specific flocculation test for the diagnosis of trichinosis by adsorbing crude larval extract onto stock bentonite. Although this test is relatively easy to read, simple to prepare, and requires only small amounts of serum, it is not widely used because the trichina extract may deteriorate when stored over long periods of time,[1] thus making it impractical to produce it in large amounts in a central laboratory for general distribution to smaller laboratories.

The present series of experiments was designed to test whether the acid soluble protein fraction antigen can be used effectively in the rapid flocculation test. An attempt was also made to compare the reaction of the purified and crude antigens with serums from man, as well as from lower animals, in order to test the hypothesis that species differences may exist in the production of demonstrable antibodies to *Trichinella* larvae.

MATERIALS AND METHODS

The crude larval antigen was prepared with strict adherence to the method described by Bozicevich, *et al.* (1951) with the exception that 5 ml. instead of 2 ml. of settled larvae were used per 100 ml. of antigen. Since only a constant maximum amount of antigen can be coated onto the bentonite, it was felt that a greater concentration of antigen would not alter the results. Preliminary tests with 67 positive sera gave identical titers with antigens prepared from 2 and 5 ml. of larvae. The crude larval extract was frozen and stored until used, which was never later than 4 months after its preparation.

The acid soluble protein extracts of trichina larvae was prepared according to the method described by Melcher (1943). Aliquots of the finished product, in the form of a powder, were sealed in different vials and stored under refrigeration at 4°C. Solutions were made every two or three months by dissolving 200 mg. of powdered extract in 10 ml. of normal saline. This, also, is assumed to be an excess of antigen to come in contact with the bentonite particles.

The flocculation test was performed according to the instructions published by

Received for publication, March 28, 1955.

[1] In our laboratory, some batches have retained their antigenic properties when frozen and stored at −10°C. for 3 years, whereas others have lost their potency in only 6 months.

Bozicevich, *et al.* (1951). Certain arbitrary standards were chosen throughout the series of experiments. A stock bentonite suspension was used within 3 months from the time of preparation. The stock bentonite-antigen preparation was used only for two months and then discarded, and the test antigen was never stored for longer than 1 month. Tween-80 solution was prepared fresh for each series of tests. The antigens were adjusted with Tween-80 to 1 plus or less flocculation and titrated against 4 positive and 2 negative serums. At least one of the positive serums chosen possessed a very low titer. Only those reactions which were read as 3 or 4 plus with serums diluted 1 : 5 were recorded as positive. Both positive and negative controls were included in each day's run of tests. All the serums were inactivated before the flocculation tests were performed with both antigens.

Rats, rabbits, and hogs were used as experimental animals. The rats used were young adults. After having been inoculated intragastrically with 3,000 washed larvae each, they served to supply fresh larvae for the preparation of antigens and for the infection of the rabbits and hogs. The larvae were separated from the muscles by artificial digestion in a pepsin-hydrochloric acid mixture not less than 30 days after the rats had been infected. The rabbits included in this series, 26 in all, were adults varying in weight from 2,000 to 4,000 grams at the beginning of the experiments. They were given, intragastrically, variable numbers of washed larvae. The 15 pigs included in this series were 4 to 7 weeks old at the beginning of the experiments. They were reared in separate pens under controlled experimental conditions at the University of Georgia, School of Veterinary Medicine, and were infected, by mouth, with variable doses of larvae. All animals were kept on standard diets.

The serums were obtained from the rabbits and the hogs by bleeding them just before inoculation and every week thereafter up to the end of the experiments. The rabbits were bled routinely from the marginal ear vein. Occasionally, when large amounts of serum were needed, they were bled from the heart. The hogs were bled from the vena cava.

A total of 1,121 serums from humans suspected of being infected with *Trichinella* were received in the Serology Laboratory of CDC and, to obviate bias, were coded before being sent to the Parasitology Laboratory for the flocculation tests. Some of the human serums had received merthiolate before being shipped to the CDC laboratories; others arrived with obvious signs of bacterial contamination. However, neither merthiolate in the amounts used for serum preservation nor the bacterial contamination seemed to interfere with the flocculation reaction with either one of the two antigens tested. All the serums were also examined by the complement fixation test using, as antigen, the whole larval extract. A direct comparison of the results obtained in human serums by the use of the flocculation and the complement fixation tests will be reported elsewhere.

RESULTS

1. *Flocculation Tests with Rabbit Serum*

Of the rabbits included in this experiment, 10 were inoculated with a single dose of variable numbers of infective trichina larvae, 13 received multiple infections and 3 were used as uninfected controls. The serums from these rabbits gave negative flocculation reactions with both antigens before and 1 week after inoculation

TABLE I.—*Comparison of whole larval antigen and acid soluble protein fraction antigen in the flocculation tests on serums from rabbits experimentally infected with* Trichinella spiralis

Rabbit no.	Larvae inoculated X 1000	Time, in weeks, from ingestion of larvae																															
		0		1		2		3		4		.....	13		14		15		16		17		18		19		20		21		22		23	
		Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	.....	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf
S-4	20	–	–	–	–	–	–	+	+	+	+																						
S-32	20	–	–	–	–	–	–	–	–	+	+	.....	+	+	+	+	+	+	Nd	Nd	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-34	20	–	–	–	Nd	–	–	–	–	+	+	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-24	40	–	–	–	–	–	–	+	+	+	+																						
S-53	80	–	–	–	–	–	–	+	+	+	+																						
O-177	90	–	–	–	–	+	+	+	+	+	+	.....	+	+	+	+	+	+	+	+	+	+	+	+										
H-22	100	–	–	–	–	–	–	–	–	–	–	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
H-23	100	–	–	–	–	–	–	+	+	+	+	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
S-16	100	–	–	–	–	+	Nd	+	+	+	+																						
S-13	200	–	–	–	–	–	+	+	+	+	+																						
S-1	5 ; 7 ; 20 ; 5 ; 25 ; 400	–	–	–	–	–	–	–	–	+	+																						
S-3	5 ; 7 ; 20 ; 5 ; 25 ; 400	–	–	–	–	–	–	–	–	+	+																						
S-5	20 ; 20 ; 20 ; 90	–	–	–	–	–	–	+	+	+	+	.....	+	+	+	+	+	+	+	+	–	–	–	+	–	+	–	–	–	+	–	+	+	+
S-6	40 ; 20 ; 20 ; 90 ; 235	–	–	–	–	–	–	+	+	+	+	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
S-7	40 ; 20 ; 20 ; 90	–	–	–	–	–	–	+	+	+	+	.....	–	–	–	–	–	–	+	+	–	–	+	+	–	–	+	+	+	+	+	+	+	+
S-8	60 ; 20 ; 20	–	–	–	–	–	–	+	+	+	+																						
S-9	50 ; 20 ; 20 ; 90 ; 235	–	–	–	–	–	–	+	+	+	+	.....	+	+	Nd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-17	100 ; 235	–	–	–	–	+	+	+	+	+	+	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-25	40 ; 235	–	–	–	–	–	–	+	+	+	+	.....	+	+	+	+	+	+	+	+	+	+												
S-27	60 ; 235	–	–	–	–	–	–	+	+	+	+	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-29	80 ; 235	–	–	–	–	–	–	+	+	+	+	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-38	200 ; 235	–	–	–	Nd	–	–	–	–	+	+	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-39	200 ; 235	–	–	–	Nd	–	–	–	–	+	+	.....	+	+	+	+	+	+	+	+	+	+												
C-1	0	–	–	–	–	–	–	–	–	–	–	.....	–	–																				
C-2	0	–	–	–	–	–	–	–	–	–	–	.....	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C-3	0	–	–	–	–	–	–	–	–	–	–	.....	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Wl = Whole larval antigen.
Pf = Acid soluble protein fraction antigen.
Nd = Not done.

(Table I). The three uninfected controls were negative throughout the experiment. On the second week, two of the 23 inoculated rabbits (S-17 and O-177) were positive with both antigens; one (S-16) was positive with the whole larval antigen and was not tested with the purified protein and one (S-13) was negative with the whole larval antigen and positive with the purified fraction. On the fourth week only one infected rabbit (H-22) was still negative and from the fifth to the 12th week they were all positive with both antigens. From the 13th week to the end of the experiment, reversal of results from positive to negative and back to positive occurred in two rabbits (S-5 and S-7).

All serums from the infected rabbits were serially diluted; 1 : 5, 1 : 10, 1 : 20, etc. Positive serums were titrated out until a negative reaction was obtained. The highest titers reported with either antigen were reached around the eighth or ninth week following inoculation. The highest recorded was 1 : 2,560. A striking similarity of titers was observed in all animals with both antigens throughout the experiment. In those few instances in which the titer with the two antigens was different, the protein fraction antigen was one dilution higher. At the end of the experiment, the rabbits were sacrificed. Numerous *Trichinella* larvae were found in the diaphrams of all the experimentally infected animals, whereas none were found in the controls.

2. Flocculation Tests with Hog Serums

In this experiment, 11 hogs were inoculated with single doses of infective larvae varying from a few hundred to 25,000 per animal. Four hogs were used as uninfected controls. A perusal of Table II shows that all the serums from the hogs gave negative flocculation reactions with both antigens before and 1 week after inoculation. On the second week, two hogs (1 and 5) gave positive reactions with both antigens. All the hogs whose serums ever became positive had given a positive reaction by the fifth week. Beginning with the seventh week, some hogs began giving negative reactions and by the 14th week, all the infected animals gave negative flocculation reactions with both antigens. Similar results were obtained with the two antigens. However, under the conditions of this experiment, in several hogs infections were detected at an earlier stage and for a longer period of time with the protein fraction antigen than with the whole larval antigen. The uninfected controls, as well as 4 of the hogs which received a very light infection, gave negative reactions with both antigens throughout the experiment. One hog (11) which was inoculated with a small number of larvae gave a positive reaction with the protein fraction on the fifth, sixth, and ninth week from the time of the inoculation and was negative with both antigens throughout the rest of the experiment. At the end of the experiment, 30 grams of diaphragm muscle from each animal were digested with artificial gastric juice by the usual technique. Trichina larvae were observed in all of the hogs experimentally infected, whereas no larvae were found in the controls.

3. Flocculation Tests with Human Serums

A total of 1,121 human serums from patients suspected of having become infected with trichinosis were examined by the flocculation test, with both antigens. Of these, 726 serums gave negative reactions and 359 serums gave positive reactions

TABLE II.—*Comparison of whole larval antigen and acid soluble protein fraction antigen in the flocculation tests on serums from swine experimentally infected with* Trichinella spiralis

Hog no.	Infective dose	Time, in weeks, from ingestion of larvae																											
		0		1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
		Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf	Wl	Pf
1	Heavy	Nd	Nd	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Nd	Nd	Nd	Nd	+	+						
3	Medium	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	+	–	+	–	–
4	Medium	–	–	–	–	–	–	–	–	–	+	Nd	Nd	+	+	+	+	–	+	–	+	–	+	–	+	–	–	–	–	–	–
5	Medium	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	Nd	Nd	–	+	–	+	–	+	–	–
6	Medium	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	Nd	Nd	–	+	–	+	–	+	–	–
7	Medium	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	Nd	Nd	–	+	–	+	–	+	–	+	–	+	–	–
11	Light	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
12	Light	–	–	–	–	–	–	–	–	–	–	Nd	Nd	–	–	–	–	–	–	–	–	–	–	Nd	Nd	Nd	Nd	–	–	–	–
13	Light	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	Light	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	Light	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Nd	Nd	–	–	–	–	–	–	–	–	–	–		
2	None	Nd	Nd	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Nd	Nd	Nd	Nd	–	–						
8	None	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Nd	Nd	–	–	–	–	–	–	–	–	–	–	–	–
9	None	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	None	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Wl = Whole larval antigen.
Pf = Acid soluble protein fraction antigen.
Nd = Not done.

with both antigens (Table III). Of the 36 serums behaving differently with the 2 antigens, 21 gave negative reactions with the protein fraction and positive with the whole larval antigen and 15 gave positive reactions with the protein fraction

TABLE III.—*Comparison of whole larval antigen and acid soluble protein fraction antigen in the flocculation test on human serums*

		Whole larval antigen		
		Positive	Negative	Total
Protein fraction antigen	Positive	359	15	374
	Negative	21	726	747
	Total	380	741	1,121

and negative with the whole larval antigen. These differences occurred exclusively among serums which were positive with the other antigen at a dilution of 1 : 5.

Detailed clinical histories, tentative physician's diagnoses, and the results of complement-fixation tests[2] were available on 9 patients with contrasting serological results. Of these, five were positive only with the whole larval antigen and four were positive only with the protein fraction antigen. Two of the five serums of the former group and three of the four serums of the latter group were positive by the complement-fixation test. All of the five individuals of the former group had recent histories of myositis and 2 of these had recent histories of sub-orbital edema. The tentative diagnosis made by the physician on clinical grounds was trichinosis for three of the five of the former group and for three of the four of the latter group.

DISCUSSION

The results of the present investigation, in general, indicate a striking similarity of results by using in the flocculation test a crude whole larval extract or an acid soluble protein fraction. Neither antigen was consistently more sensitive than the other in detecting the appearance of circulating antibodies following the ingestion of *Trichinella* larvae. The acid soluble fraction, however, occasionally seemed to confer to the flocculation test a somewhat greater sensitivity than the whole larval crude extract. A much larger series of tests, using both antigens, must be performed before deciding whether or not this occasional greater sensitivity is an accidental finding.

The striking similarity of results with both antigens furnishes indirect evidence in favor of Melcher's contention (1943) that the acid soluble protein fraction is the fraction which is responsible for both precipitating and skin test activity, and extends it to include activity in the flocculation test. In fact, it is possible that the bentonite in the flocculation test may act as a purifier or a selector which absorbs from the whole larval extracts the same protein fraction which is chemically separated by the Melcher's technique. Attention should be called to the fact that the products used in these tests are probably not pure antigens, but relatively crude extracts of heterogenous composition and represent, in reality, a mosaic of antigens.

The two antigens employed in the flocculation test reacted similarly with serums from man and lower animals. This finding is in variance with those reported by Frisch and his co-workers (1946) who found that the acid-soluble fraction was distinctly less reactive than the crude extract when tested with humans.

[2] Performed by the Serology Section of the Communicable Disease Center, Public Health Service.

They explained the different results obtained by Melcher (1943) in rabbits with those obtained by the above authors in humans as an indication of "species differences to immunization with larvae." No such evidence was encountered in the present series of experiments.

Of immediate practical importance is the observation that the acid-soluble protein fraction produced a flocculation test easier to read and to interpret than the flocculation test previously described with the whole larval antigen. The flocculating particles with the protein fraction antigen were more discrete and more sharply defined. Also, while the whole larval antigen could not always be kept over long periods of time without altering its antigenic properties, the protein fraction antigen could be kept over long periods of time, in powdered form, even without freezing. This permits it to be standardized and stored for general distribution to small laboratories to be used whenever the need arises.

In general, the flocculation tests, with both antigens, gave consistently satisfactory results with serums from man and lower animals. No evidence of flocculation was ever noted in any of the negative animal control serums. Even if 2-plus reactions, instead of only 3-plus or 4-plus, had been interpreted as positives, no false positives would have been recorded in these experiments. However, false negatives were observed, especially with serums from hogs which had received mild infections. Also, reversals of results from positive to negative and back to positive were occasionally observed on specimens obtained on consecutive weeks from the same animals, both with hogs and rabbit serums. These findings are in agreement with those previously reported (Norman, *et al.*, 1955; Thomas, *et al.*, 1953).

SUMMARY

An acid soluble protein fraction antigen and a whole larval antigen were used in the flocculation test for the diagnosis of trichinosis in human, hog, and rabbit serums. The results obtained with the two antigens were very similar. The acid soluble fraction, however, could be stored indefinitely, seemed to confer upon the flocculation test a somewhat greater sensitivity, and produced a test easier to read and to interpret. No consistent differences in the reactions were obtained from serums of man and of the lower animals.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. A. W. Donaldson, of the Communicable Disease Center, for his interest and cooperation in this study. Thanks are due to Dr. J. Schubert, also of the Communicable Disease Center, for courteously allowing us access to the results of the complement-fixation tests on human serums.

LITERATURE CITED

BOZICEVICH, J., TOBIE, J. E., THOMAS, E. H., HOYEM, H. M., AND WARD, S. B. 1951 A rapid flocculation test for the diagnosis of trichinosis. Public Health Reports **66**: 806–814.

FRISCH, A. W., WHIMS, C. B., AND OPPENHEIM, M. 1946 Immunologic reactions in trichinosis with purified antigens. Am. J. Clin. Path. **17**: 29–34

MELCHER, L. R. 1943 An antigenic analysis of *Trichinella spiralis*. J. Inf. Dis. **73**: 31–39.

NORMAN, L., SADUN, E. H., REDDING, R. W., AND COOPERRIDER, D. E. 1955 Flocculation test in sera from hogs experimentally and naturally infected with *Trichinella spiralis*. J. Parasit. **41**: 162–166.

THOMAS, E. H., BOZICEVICH, J., AND HOYEM, H. M. 1953 Flocculation reactions in rabbits experimentally infected with *Trichinella spiralis*. J. Inf. Dis. **92**: 89–96.

THE ANTIGENIC ROLE OF THE EXCRETIONS AND SECRETIONS OF *TRICHINELLA SPIRALIS* IN THE PRODUCTION OF IMMUNITY IN MICE[1]

CHARLES H. CAMPBELL[2]

Department of Parasitology, University of North Carolina, Chapel Hill, North Carolina

During the last two decades a great deal of experimental work has been concerned with immunological studies on trichinosis. Owing to the wide host range of *Trichinella spiralis,* these studies have been performed in a variety of experimental animals. Following one or more immunizing infections, immunity to a challenging infection has been demonstrated in the rat (McCoy, 1931, 1932), rabbit (Oliver-Gonzalez, 1940), and guinea pig (Roth, 1939). Since the work of Culbertson (1942) with mice, attention has been directed toward the use of this host in such studies. Much of the work performed with mice has been reviewed recently by Larsh (1953a).

Although the mechanism of the immune response to infection with *T. spiralis* is not known definitely, present evidence gives strong support to the theory of Taliaferro (1940) that the immune response is due primarily to the action of antibodies with secondary cellular cooperation as described by Taliaferro and Sarles (1939) in their immunity studies of the nematode, *Nippostrongylus muris.* Recent studies with *T. spiralis* giving strength to this hypothesis have shown that the immunity in old mice is of a general nature (Larsh, 1953b) and that an inflammatory response in the intestine precedes the loss of adult worms (Larsh and Race, 1954). The latter authors suggest that *T. spiralis* adults are partially or completely immobilized by antibodies after which phagocytic cells attack the worms and complete their destruction or make conditions in the intestine unfavorable so that they pass rapidly from the host.

On the basis of studies with many nematodes, it has become increasingly evident that the excretions and secretions of parasitic worms act as antigens in the stimulation of the immune response(s) in a host. This evidence has consisted largely of the demonstrations that precipitates are formed around the various body openings of worms incubated in homologous antiserum (Taliaferro and Sarles, 1939; Oliver-Gonzalez, 1940, 1943; Otto, 1940; Mauss, 1941; Otto, Schugam, and Groover, 1942; Sadun, 1949; Smith, 1953). The only known study, however, involving the use of excretions and secretions in an attempt to produce artificially acquired immunity is that of Thorson (1953), who found that injections of the excretions and secretions of *Nippostrongylus muris* stimulated a protective immune response to this parasite in rats.

Received for publication, April 18, 1955.

[1] Condensed from a thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the School of Public Health.

The author wishes to express his sincere appreciation to Dr. John E. Larsh, Jr., under whom the work was carried out, for his helpful criticisms and suggestions throughout the study and in the preparation of the manuscript. The author also is indebted to Dr. W. W. Cort for aid in preparation of the manuscript, and to Dr. B. G. Greenberg and Dr. Harry Smith, Jr., for guidance in the statistical interpretation of the data.

[2] Present Address: Department of Microbiology and Immunology, State University of New York, College of Medicine, New York City.

In an effort to add to the understanding of the mechanism of immunity to *T. spiralis*, the present study was designed to determine the immune effects produced in mice by injections of the excretions and secretions of this parasite. The study was based on the following hypothesis: (1) immunity to *T. spiralis* is primarily of a humoral nature involving the production of protective antibodies in response to antigenic stimulation, and (2) the excretions and secretions of *T. spiralis* are important antigenic agents in the stimulation of this immunity.

MATERIALS AND METHODS

White mice of the stock strain used for many years in the work of this Department (Larsh and Kent, 1949; and others) were employed as the experimental animal in this study.

The strain of *T. spiralis* used in the present study was that employed in all previous work in this laboratory. It was maintained originally in rats, but in recent years mice have been used for this purpose. The larvae were isolated, standardized, and administered, and counts of adults and larvae were made as described previously by Larsh and Kent (1949) and Larsh *et al.* (1953). In one experiment adult female worms were measured with the aid of a microscope fitted with a camera lucida. An outline of the worm being measured was drawn, the length of the outline was determined with a map-measuring instrument, and by means of the magnification factor of the microscope and camera lucida the actual length of the worm was calculated.

The antigen selected for this study was associated with the excretions and secretions of *T. spiralis* larvae. Hereafter this antigen will be called the ES antigen. It was collected by incubating the larvae in a nutrient fluid, after which the worms were removed and discarded.

To obtain the excretions and secretions the larvae were isolated from infected mice, and the total number was estimated by dilution counts. They were washed repeatedly by allowing them to settle through six tubes of warm (37°C.) sterile 0.85 percent saline. After the larvae had settled to the bottom of one tube, they were transferred to the next tube by means of a sterile pipette, fitted with a rubber bulb. After the last washing, they were placed in warm saline contained in a 30 ml. conical centrifuge tube, and the volume of saline in this tube was adjusted, after the larvae had settled, to obtain the desired number of worms, 25,000, per 1.0 ml. This number in 1.0 ml. of saline was transferred to 50 ml. flasks containing 9.0 ml. of sterile nutrient fluid making a total volume of 10.0 ml. Thus the final concentration of larvae was 2,500 per ml. The nutrient fluid consisted of Simms $\times 6$ solution (two parts) and ox serum ultrafiltrate (one part) as described by Simms and Sanders (1942). Although these authors suggest the use of carbon dioxide and sintered glass filtration in preparing the $\times 6$ solution for tissue culture work, no carbon dioxide was used in this study and the solution was sterilized by autoclaving. Antibiotics (500 units of penicillin and 500 μg. of streptomycin per ml. of fluid) were added to inhibit bacterial contaminants.

After the larvae had been added, the flasks were stoppered with gauze-covered cotton plugs and placed in an incubator at 37°C. for five days. In preliminary experiments microscopic examination of samples of the worms incubated under these conditions revealed that viability was maintained for six to seven days as evidenced by motility. Photomicrographs of these samples revealed also that devel-

opment of the worms was occurring as shown by the appearance of sheaths and an increase in length. Control fluids, consisting of 9.0 ml. portions of nutrient fluid to which no larvae were added, were incubated at the same time. Following incubation, the contents of the flasks were poured into sterile conical centrifuge tubes, and, after settling, the larvae were removed and discarded. The few remaining larvae were concentrated by centrifugation at 2000 r.p.m. for five minutes and removed. The fluid was dispensed in two–three ml. amounts into sterile vaccine bottles closed with rubber stoppers and stored in the deep-freezer at – 10°C. until needed.

The ES antigen and the control solutions were removed from the deep-freezer and administered soon after thawing occurred. By means of a sterile one ml. tuberculin syringe and 0.5 inch 27-gauge needle, the fluid was administered subcutaneously in the inguinal region of the animal, the injections being given alternately in the right and left inguinal areas.

The following criteria of immunity were used: (1) counts of adult worms seven days after a challenging infection, or 7, 9, 11, and 13 days after a challenging infection; (2) length measurements of adult female worms; and (3) counts of larvae recovered 30 days after a challenging infection.

EXPERIMENTAL PROCEDURES AND RESULTS

Series A

Experiment I.—This experiment was designed to determine if injections of the ES antigen at three-day intervals would stimulate the production of immunity in mice.

Fifty-six male mice between two and three months old were used. Twenty-eight were given subcutaneous injections of the ES antigen, and 14 were given subcutaneous doses of the culture fluid only. A total of 0.9 ml. of ES antigen or control fluid was administered to each animal. This quantity was divided into six doses, one being given every three days as follows: 0.10 ml., 0.10 ml., 0.15 ml., 0.15 ml., 0.20 ml., and 0.20 ml. Fourteen mice, not treated, were used as non-injected controls. Two weeks after the last injection, all of the animals were given a challenging infection of 200 larvae.

Seven days after the challenging infection, the animals from which adult worms were to be recovered were sacrificed. Twenty-three days later, or 30 days post-infection, the remaining animals were sacrificed for counts of larvae.

The average numbers of adult worms recovered seven days after the challenging infection and the average numbers of larvae recovered 23 days later are recorded in Table I. The mice injected with the ES antigen had an average of 148.5 adult worms; those injected with control fluid harbored an average of 142.1 adults; and an average of 146.7 adults was recovered from the non-injected controls. While these counts of adults were quite similar, those of the larvae showed a striking difference. The antigen-injected mice harbored an average of 16,571 larvae, while those injected with the control fluid had an average of 32,325, or about the same number as the non-injected controls (33,611).

The results of this experiment were analyzed by Student's "t" test. Comparisons were made between the results of the group injected with control fluid and the non-injected group, and when no significant difference was found between them,

they were combined for comparison with the results of the group injected with the ES antigen. No significant difference was observed between the numbers of adult worms recovered from the various groups, but the numbers of larvae recovered from the antigen-injected mice were significantly smaller than the numbers from the other two groups (Table I). These findings indicate that under these experimental conditions injections of the ES antigen resulted in the production of immunity only as judged by counts of larvae.

TABLE I.—*Average number of* T. spiralis *adults recovered seven days after infection with 200 larvae and the average number of larvae recovered thirty days after infection from: (1) mice injected at three-day intervals with the ES antigen,* (2) mice injected with the control fluid, and (3) non-injected mice.*

Injected with ES antigen				Injected with control fluid				Non-injected			
No. mice	Avg. no. adults	No. mice	Avg. no. larvae	No. mice	Avg. no. adults	No. mice	Avg. no. larvae	No. mice	Avg. no. adults	No. mice	Avg. no. larvae
14	148.5	14	16,571	7	142.1	7	32,325	7	146.7	7	33,611

* Antigenic material associated with the excretions and secretions of the larvae.

Experiment II.—This experiment was performed to determine the effect(s) on immunity of more frequent injections of the ES antigen and a shorter period of time between the last injection and the challenging infection.

The same ES antigen and the same dosages were used in this experiment as in Experiment I. In this case, however, the injections were given every two days, and the challenging infection was administered seven days after the last injection. A control fluid group was not included in this experiment and those that followed, since the results of the first experiment indicated no observed effects of the fluid alone on immunity.

Counts of adults and larvae were made as in Experiment I. Again essentially no difference was observed in the numbers of adults recovered seven days after the challenging infection, whereas 30 days after infection the injected mice harbored significantly smaller numbers of larvae (avg. 22,160) than non-injected control mice (avg. 34,560).

While both of the experiments of Series A were negative in regard to the production of an immune response measured by the number of adult worms recovered seven days after infection, an observation was made which seemed to be important. On necropsy the small intestines of the injected animals were found to be distended and to contain a watery, purulent substance, whereas this condition was not observed in the control animals. It is known that several days before adult worms are lost from immunized old mice, a severe inflammatory response, believed to be important in eliminating the worms, occurs in the anterior small intestine. The above observation indicated that a similar response might have been occurring in the small intestines of the mice injected with the ES antigen. If this were true, a loss of adult worms could be expected several days after the appearance of this response. The earlier loss of adult worms from the injected mice and the consequent interruption of the reproductive phase of the worms would explain, at least in part, the finding of smaller numbers of larvae in the antigen-injected animals.

Series B

Experiment I.—This experiment was designed to determine if adult worms are lost more rapidly from mice injected with the ES antigen than from non-injected mice.

Fifty female mice, approximately four months old at the beginning of the experiment, were separated into two groups of 25 mice each. One of these groups received the usual six injections at three-day intervals of the ES antigen, and the other group of animals received no injections.

Two weeks after the last injection, all of the mice were given a challenging infection of 200 *T. spiralis* larvae. On the 7th, 9th, 11th, and 13th day after infection, five mice from each group were selected at random and sacrificed for counts of adult worms. On the 30th day after infection, the remaining five mice in each group were sacrificed for counts of larvae.

The average numbers of adult worms recovered 7, 9, 11, and 13 days after infection and the numbers of larvae recovered 30 days after infection are recorded in Table II. At 7, 9, 11, and 13 days, the average numbers of adults recovered from

TABLE II.—*Average number of* T. spiralis *adults recovered 7, 9, 11, and 13 days after infection with 200* T. spiralis *larvae and the average number of larvae recovered 30 days after infection from: (1) mice injected at three-day intervals with the ES antigen and (2) from non-injected mice.*

Days after injection	Injected with ES antigen		Non-injected	
	No. mice	Avg. no. adults	No. mice	Avg. no. adults
7	5	105.8	5	113.6
9	5	63.8	5	114.4
11	5	71.0	5	87.0
13	5	8.4	5	44.6
		Avg. no. larvae		Avg. no. larvae
30	5	8,424	5	26,864

the antigen-injected mice and the control mice, respectively, were: 105.8 and 113.6, 63.8 and 114.4, 71.0 and 87.0, and 8.4 and 44.6.

An analysis of variance was performed with the data from the counts of adults, and a significant difference was found to exist between the results of the various days. From this information it was concluded that immunity stimulated by the challenging infection was responsible, in part, for the loss of worms between the 7th and 13th days. The loss of worms from the controls is ascribed entirely to this response. The analysis of variance also revealed that there was a significant difference between the results of the mice injected with the ES antigen and the non-injected control mice, thus indicating that injections of the antigen stimulated an immune response resulting in an earlier loss of worms from the injected animals.

Averages of the estimated numbers of larvae recovered from five mice from each of the groups also are shown in Table II. The mice injected with the ES antigen harbored an average of 8,424 larvae, whereas the control mice had an average of 26,864 larvae. These counts were analyzed by Student's "t" test. As would be expected from the results of the Series A experiments, a significant difference was found between the results of the two groups.

Experiment II.—This experiment was designed as a repetition of Experiment I

of this Series. In the present case, older mice were used, since it is known that they develop a more striking immunity than young mice. Also, in designing the present experiment the possibility was considered that in the previous experiments the female worms were unable to develop normally in the antigen-injected mice and thus failed to give off the usual numbers of larvae.

Forty female mice, six months old at the beginning of the experiment, were divided into two groups of 20 mice each. One group received injections of the ES antigen, while the other group was used for non-injected controls. The same schedule was used in this work as in the previous experiment. In addition to total worm counts, samples of female worms were taken for measurements of length seven days post-infection to determine the effect, if any, of the immune response on the size of the worms. From each of the two groups, samples of 50 female worms were measured.

The average numbers of adult worms recovered and the average lengths of the 50 female worms measured from each group are recorded in Table III. At seven days after the challenging infection, the mice injected with the ES antigen harbored an average of 91.8 adults of which the females averaged 2.37 mm. in length, whereas the non-injected mice harbored an average of 109.6 adults of which the females averaged 2.73 mm. At 9, 11, and 13 days, the average numbers of adults recovered from the antigen-injected mice and the controls, respectively, were: 47.8 and 98.2, 15.0 and 74.8, and 11.6 and 35.4.

An analysis of variance was performed on the counts of the adult worms, and the results were essentially the same as for Experiment I of this Series. Again the difference between the two groups was found to be significant, thus indicating that the ES antigen had stimulated an immune response which resulted in a more rapid loss of worms.

The results of the measurements of length of the female worms recovered from the two groups seven days after infection (Table III) were analyzed by Student's

TABLE III.—*Average number of* T. spiralis *adults recovered 7, 9, 11, and 13 days after infection with 200 larvae from: (1) old mice injected at three-day intervals with the ES antigen and (2) old non-injected mice. (The average lengths of adult females 7 days after infection are also listed.)*

Days after infection	Injected with ES antigen		Non-injected	
	No. mice	Avg. no. adults	No. mice	Avg. no. adults
7	5	91.8 Avg. length females 2.37 mm.	5	109.6 Avg. length females 2.73 mm.
9	5	47.8	5	98.2
11	5	15.0	5	74.8
13	5	11.6	5	35.4

"t" test. The worms from the mice injected with the ES antigen were found to be significantly shorter (approximately 15 percent) than those from the non-injected mice. It was concluded, therefore, that injections of the ES antigen, under these experimental conditions, stimulated an immune response inhibiting the growth of the female worms.

DISCUSSION

Consideration of the nature of the antigen is of great importance in interpreting an immune response following its injection into experimental animals. Since the

present study was concerned with the immune response following injections of the excretions and secretions of *T. spiralis*, it was necessary to exclude other antigenic fractions of the worms from the experimental antigen. In preparing the ES antigen, therefore, microscopic examinations were made daily on samples of the worms being incubated in the nutrient fluid to determine if the worms were still viable. This examination was necessary, owing to the fact that dead worms on disintegrating might release various antigens which would invalidate the study. Since the worms remained viable during the period of incubation, it was concluded that antigenic portions of the internal tissues were not present in the preparation.

The present experiments demonstrated that injections into mice of the excretions and secretions of *T. spiralis* stimulated the production of an immune response. While this response did not affect the number of adult worms recovered seven days after a challenging infection, it did result in decreased numbers of larvae recovered 30 days after the infection. Effects on the adult worms were demonstrated, however, by the more rapid loss of adults and the smaller size of adult females in mice injected with the antigen as compared with non-injected control mice. It is clear that the immunity produced artificially by injections of the ES antigen is much less striking than that observed following natural infections. This is in keeping with previous work showing that artificially produced active immunity does not compare in degree with active immunity acquired naturally. Both quality and quantity of the antigen used in such studies, no doubt, are involved. It is important to recall that in the present experiments it was not possible to measure the immunity by the criterion (number of adults present seven days after the challenging infection) usually used in work of this type. Thus, in other studies on active immunity produced artificially it may be necessary to devise more delicate means for demonstrating the immune response(s).

While results of the counts of the larvae suggest that the immune response affected the larvae, previous studies of the response following immunizing infections have indicated that it is effective primarily against the intestinal stages of the parasite (Culbertson and Kaplan, 1938; Culbertson, 1942; and others). While in the present case, it is not possible to rule out immune effects directed primarily against the migrating larvae, there is no evidence from the many studies on *T. spiralis* that protective antibodies are formed against the larvae in the circulatory system and/or in the muscles of the host. On the contrary, there is evidence that antilarval antibodies are non-protective in action (Oliver-Gonzalez, 1941). It is suggested, therefore, that the smaller numbers of larvae recovered from the antigen-injected mice were due to an interruption of the reproductive potential of the adult females as evidenced by the more rapid loss of worms and the stunting of the adult females in those animals. The demonstration at seven days of a stunting effect on the females is in agreement with the observations of Rappaport and Wells (1951), who noted this effect in mice given one stimulating infection with *T. spiralis*. Likewise, interference with the reproductive potential due to the stunting of female worms is in keeping with observations made in *Nippostrongylus muris* infections in which the stunting of adult female worms in immunized rats is associated with a decrease in the number of eggs released (Schwartz, Alicata, and Lucker, 1931; Chandler, 1936).

The demonstration that excretions and secretions of *T. spiralis* larvae serve

as an effective antigen in stimulating immune responses in mice adds to the direct evidence presented by Thorson (1953). Working with *Nippostrongylus muris* he found that injections of the excretions and secretions of the infective larvae stimulated a protective immune response in rats. Thus for *T. spiralis* in mice and *N. muris* in rats there is proof for the generally accepted view that excretions and secretions of parasitic worms are important antigenic materials stimulating immune responses in the host.

SUMMARY

The excretions and secretions of *Trichinella spiralis* were collected by incubating the infective larvae in a nutrient fluid for five days after which the worms were discarded. Injections of the excretions and secretions stimulated the production of an immune response as evidenced by a more rapid loss of adult worms, stunting of adult female worms, and smaller numbers of larvae recovered from injected mice as compared with control mice.

REFERENCES CITED

CHANDLER, A. C. 1936 Studies on the nature of immunity to intestinal helminths. III. Renewal of growth and egg production in Nippostrongylus after transfer from immune to non-immune rats. Am. J. Hyg. **23**: 46–54.

CULBERTSON, J. T. 1942 Active immunity in mice against *Trichinella spiralis*. J. Parasit. **28**: 197–202.

——— AND KAPLAN, S. S. 1938 A study upon passive immunity in experimental trichiniasis. Parasitology **30**: 156–166.

LARSH, J. E., JR. 1953a A review of recent immunological studies on trichiniasis in mice. Thapar Commemoration Volume, Department of Zoology, University of Lucknow, Lucknow, India. pp. 159–172.

———1953b Studies in old mice to test the hypotheses of local and general immunity to *Trichinella spiralis*. J. Infect. Dis. **93**: 282–293.

——— AND KENT, D. E. 1949 The effect of alcohol on natural and acquired immunity of mice to infection with *Trichinella spiralis*. J. Parasit. **35**: 45–53.

———, MCKENZIE, J. W., GREENBERG, B. G., AND CAMPBELL, C. H. 1953 A comparison in mice of the infectivity of *Trichinella spiralis* larvae in normal saline solution and in a solution of nutrient broth and gelatin. J. Parasit. **39** (4-Suppl.): 41–42.

——— AND RACE, G. J. 1954 A histopathologic study of the anterior small intestine of immunized and non-immunized mice infected with *Trichinella spiralis*. J. Infect. Dis. **94**: 262–272.

MCCOY, O. R. 1931 Immunity of rats to reinfection with *Trichinella spiralis*. Am. J. Hyg. **14**: 484–494.

———1932 Experimental trichiniasis infections in monkeys. Preliminary paper. Proc. Soc. Exper. Biol. and Med. **30**: 85–86.

MAUSS, E. A. 1941 The serum fraction with which anti-trichinella (*Trichinella spiralis*) antibody is associated. Am. J. Hyg. **34**: 73–80.

OLIVER-GONZALEZ, J. 1940 The *in vitro* action of immune serum on the larvae and adults of *Trichinella spiralis*. J. Infect. Dis. **67**: 292–300.

———1941 The dual antibody basis of acquired immunity in trichinosis. J. Infect. Dis. **69**: 254–270.

———1943 Antigenic analysis of the isolated tissues and body fluids of the roundworm, *Ascaris lumbricoides* var. *suum*. J. Infect. Dis. **72**: 202–212.

OTTO, G. F. 1940 A serum antibody in dogs actively immunized against the hookworm, *Ancylostoma caninum*. Am. J. Hyg. **31** (Sect. D): 23–27.

———, SCHUGAM, N. J., AND GROOVER, M. E. 1942 A precipitin reaction resulting from *Necator americanus* larvae in sera from hookworm-infected individuals. Proc. Helminthol. Soc. Wash. **9**: 25–26.

RAPPAPORT, I. AND WELLS, H. S. 1951 Studies in trichinosis. I. Immunity to reinfection in mice following a single light infection. J. Infect. Dis. **88**: 248–253.

ROTH, H. 1939 Experimental studies on the course of trichina infection in guinea pigs. III. Immunity of guinea pigs to reinfection with *Trichinella spiralis*. Am. J. Hyg. **30** (Sect. D): 35–64.

SADUN, E. H. 1949 The antibody basis of immunity in chickens to the nematode, *Ascaridia galli*. Am. J. Hyg. **49**: 101–116.

SCHWARTZ, B., ALICATA, J. E., AND LUCKER, J. T. 1931 Resistance of rats to superinfections with a nematode, *Nippostrongylus muris*, and an apparently similar resistance of horses to superinfection with nematodes. J. Wash. Acad. Sci. **21**: 259–261.

SIMMS, H. S. AND SANDERS, M. 1942 Use of serum ultrafiltrate in tissue cultures for studying deposition of fat and for propagation of viruses. Arch. Path. **33**: 619–635.

SMITH, P. E. 1953 Life history and host-parasite relations of *Heterakis spumosa*, a nematode parasite in the colon of the rat. Am. J. Hyg. **57**: 194–221.

TALIAFERRO, W. H. 1940 The mechanism of acquired immunity in infections with parasitic worms. Physiol. Rev. **20**: 469–492.

——— AND SARLES, M. P. 1939 The cellular reactions in the skin, lungs, and intestines of normal and immune rats after infection with *Nippostrongylus muris*. J. Infect. Dis. **64**: 157–192.

THORSON, R. E. 1953 Studies on the mechanism of immunity in the rat to the nematode, *Nippostrongylus muris*. Am. J. Hyg. **58**: 1–15.

THE TAXONOMY AND BIOLOGY OF *EPITEDIA WENMANNI* (ROTHSCHILD, 1904) AND *E. TESTOR* (ROTHSCHILD, 1915) (HYSTRICHOPSYLLIDAE: SIPHONAPTERA)

ALLEN H. BENTON
New York State College for Teachers
Albany, New York

Recently Hopkins (1954) described the previously unknown male of *Epitedia testor* (Rothschild, 1915), and suggested that this form probably represented a sub-species of *E. wenmanni* (Rothschild, 1904). Since Hopkins' statement was based on a relatively small number of specimens, the present study was designed to check its validity, and, if there proved to be two forms, to determine their distribution. In the process of examining several hundred specimens, some interesting data were accumulated in regard to host distribution and seasonal distribution.

I am indebted to the following individuals for the loan of personal collections or of institutional collections under their care: O. L. Cartwright, U. S. National Museum; P. J. Darlington, Museum of Comparative Zoology; Henry Dietrich, Cornell University; Richard Eads, Texas State Department of Health; John M. Geary, U. S. Air Force; G. P. Holland, Canada Department of Agriculture; E. W. Jameson, Jr., University of California; William Jellison, Rocky Mountain Laboratory; James N. Layne, Southern Illinois University; W. F. Rapp, Jr., Department of Health, State of Nebraska; Orren Royal, Birmingham, Alabama; F. G. A. M. Smit, British Museum; Harold Stark, U. S. Public Health Service; Robert Traub, Army Medical Center; and G. P. Wallace, Carnegie Museum. I am especially grateful to G. H. E. Hopkins of the British Museum, who has given freely of assistance during the study, and has critically reviewed the manuscript.

MATERIALS AND OBSERVATIONS

Material available for this study has included 544 specimens (238 males, 306 females) from 36 states, the District of Columbia, and ten Canadian provinces. It was evident at an early stage of the study that two distinct sub-species exist, as indicated by Hopkins (1954). The characteristics which separate these two forms are shown in Fig. 1. In males (Fig. 1a), the measurements designated A, B, and

Received for publication, May 11, 1955.

C are approximately equal in *E. w. wenmanni*. The ratio of C to A in this form may vary from about 70 to 110 percent. In *E. w. testor*, A is much greater than B, and two to three times as great as C. The C to A ratio is thus 30 to 50 percent. This characteristic difference is consistent in the material examined. The only areas in which intermediates appear, or in which both forms occur in collections from the same locality, are within the expected zone of intergradation, (Figure 2). Of the 238 males examined, 20 are regarded as intergrades. In these specimens, the C to A ratio is about 50 to 60 percent.

In most males, the anterior lobe of the fixed process of the clasper (L_1 in Figure 1a) greatly exceeds the posterior lobe (L_2) in *E. w. wenmanni*, while the two are nearly equal in *testor*. This characteristic is, however, not perfectly consistent.

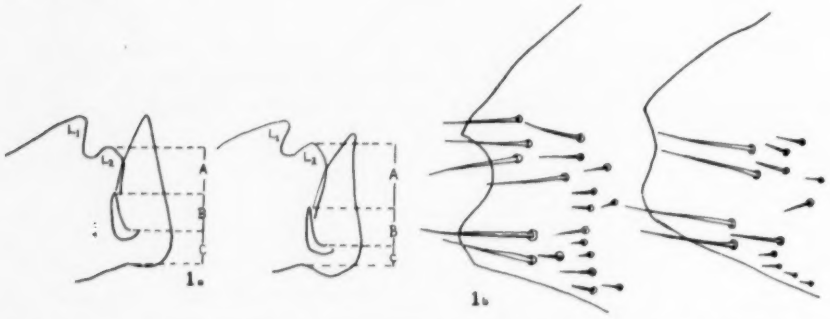


FIG. 1a. Clasper of males of *E. w. wenmanni* (left, near topotype from Blucher Hall, B. C., British Museum collection), and *E. w. testor*, (right, topotype from Lansingburgh, Rensselaer county, New York). Measurements A, B, and C, were used by Hopkins (1954) in separating these two forms.

FIG. 1b. Sternite VII of females of *E. w. wenmanni* (left, allotype from British Columbia, British Museum collection), and *E. w. testor*, (right, near topotype from Albany, Albany county, New York, New York State College for Teachers collection).

The separation of females is less distinct, depending upon minor and overlapping differences in the shape and armature of Sternite VII. In *wenmanni*, (Figure 1b), this sternite may bear from 10 to 25 bristles (average of 100 specimens, 15.1), while in *testor* the number of bristles varies from 10 to 17 (average of 50 specimens, 13.7). In *wenmanni*, the sinus of this sternite is usually deep, and the lobe acute and often hooked. The sinus in *testor* is usually shallow, and the lobe obtuse or rounded, never hooked. It is possible to separate with assurance about 80 percent of the females on morphological grounds, but females, unaccompanied by males, cannot be acecpted as indicative of the subspecies occurring in a given locality unless a considerable series is available. For this reason, records indicated in Figure 2 relate to males only.

The distribution of the two sub-species is shown in Figure 2. The northern form, *E. w. wenmanni*, ranges from the boreal region of Yukon Territory, Northwest Territory, and Labrador, south through the Hudsonian, Canadian and Transition zones of Merriam (1894). This form extends southward through the Appalachian Mountains at least to Tennessee, and through the Rocky Mountains to northern New Mexico. The type locality of this form is British Columbia.

E. w. testor reaches its northern limit in the Upper Austral zone of Merriam (1894), and extends southward through the Upper and Lower Austral zones to Alabama, New Mexico, and Arizona. The locality of this sub-species is Lansingburgh, Rensselaer county, New York.

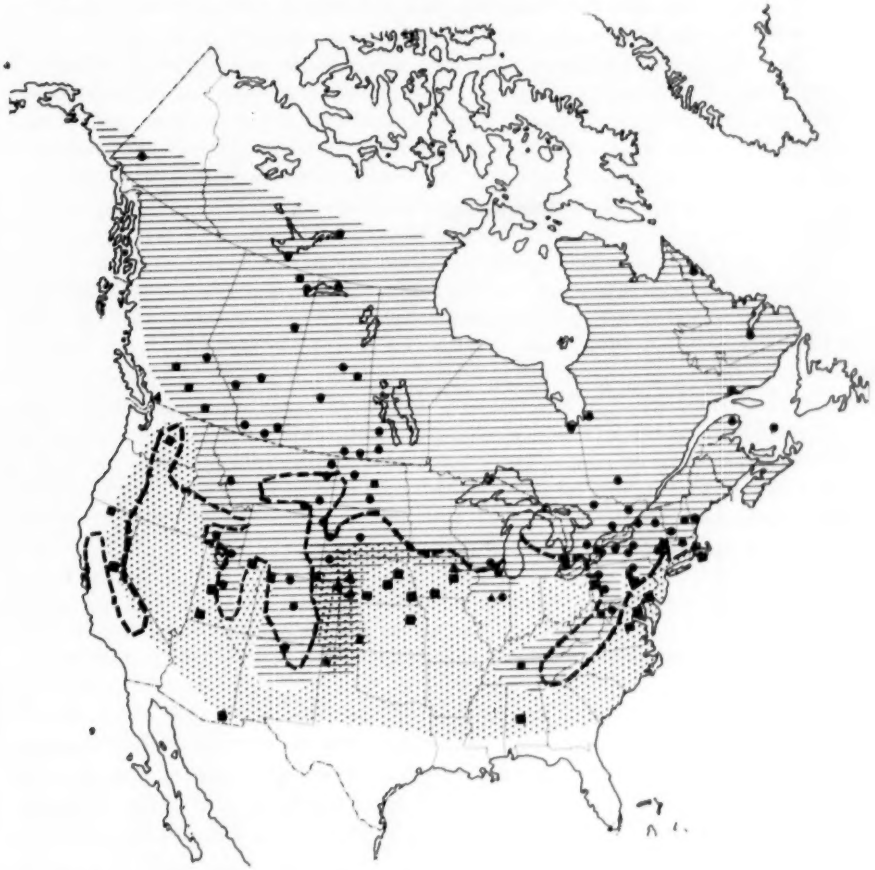


FIG. 2. Distribution of *E. w. wenmanni* and *E. w. testor*.
Symbols:
●—*E. w. wenmanni*, male specimens
■—*E. w. testor*, male specimens
▲—Intergrade, male specimens
≡—Range of *E. w. wenmanni*
⁞—Range of *E. w. testor*
- - - —Approximate boundary between the Transition and Upper Austral Zones of Merriam

The line of separation of the two sub-species follows quite closely the border between the Transition and Upper Austral zones. It is probable that the distribution of the two forms in the Rocky Mountain area is more complex than is indicated in Figure 2. Life zones in this area tend to be altitudinal rather than longitudinal, and distribution may thus be difficult to demarcate geographically.

Chapin (1919) described *Neopsylla similis* on the basis of a male from Lake Burford, Rio Arriba county, New Mexico. Jordan (1929) stated that this species was synonymous with *N. wenmanni* (Rothschild) 1904. Examination of a male from Rio Arriba county, in the collection of the Plague Suppressive Laboratory, San Francisco, indicates that the sub-species occurring in this area is *E. wenmanni wenmanni*, of which *Neopsylla similis* Chapin is a synonym.

There seems to be no question that *E. w. wenmanni* constitutes a continuous population across the entire continent, with no important regional differentiation. Specimens of *testor*, however, are lacking from much of the southern part of its range, so that this sub-species appears to be separated into three populations. In the east, the population extends from the Hudson River valley of New York to Alabama, occurring only east of the Appalachian mountains. The population from the prairie states extends eastward along the Ohio River valley to extreme western Pennsylvania, and to the Rocky Mountains on the west. In the far west, specimens have been taken from southern New Mexico and Arizona to Washington, where intergradation with *wenmanni* begins. The species is apparently not generally distributed west of the Cascade Range (Hubbard, 1947).

In the absence of striking morphological variation, it seems best to assume that these populations are actually continuous and represent a single widely distributed sub-species. Extensive collecting in areas from which specimens are lacking is needed to support or contradict this position. Small morphological variations exist among these three populations, but on the basis of specimens examined, these variations do not appear to warrant nomenclatorial recognition unless geographical separation is shown to exist.

In the prairie states, as shown in Figure 2, a wide zone of intergradation exists. In all areas of these states from which specimens have been examined, specimens of *testor* are closer to *wenmanni* than is the case elsewhere. These facts may indicate a general intermingling of the two sub-species throughout this region, where topographic barriers are slight.

As might be expected in a large series, several specimens were found which did not agree with the diagnosis of the species. In males, Sternite IX usually bears four prominent spiniforms at the tip, as well as a variable number of lateral spiniforms. In a few individuals, however, the distal spiniforms were three or five in number, and in several specimens the number was four on one side and three or five on the other.

An important characteristic of females of this species is the projection of the tail of the spermatheca into the body of this organ. Of the 306 females examined, three specimens were aberrant in this respect. Variation of this type occurs in the related genus *Catallagia*, but is apparently rare in *E. wenmanni*.

Nearly half of the specimens examined were taken from mice of the genus *Peromyscus*, or from their nests. A complete list of known hosts, however, would include nearly 50 species of mammals and at least three species of birds. Although deermice (*Peromyscus*) are undoubtedly the typical host, the frequent occurrence of these fleas in nests of wood-rats, weasels and other mammals suggests the ability to reproduce as a parasite of various mammalian species.

Hubbard (1947) suggested that adults of this species are most commonly found during the winter months. Data from this study indicate spring and fall maxima,

with smaller numbers taken in winter and summer. The low incidence in winter may be due to lack of collecting at this season. Adults are taken, however, throughout the year, and there is no significant variation in this respect between the two sub-species.

SUMMARY

Examination of 544 specimens of *Epitedia wenmanni* indicates that two subspecies exist. *E. w. wenmanni* is the more northern form, and is transcontinental in distribution. *E. w. testor* appears to have a similarly extensive range south of that of *wenmanni*. Gaps in the known distribution of this form, coupled with small morphological differences, indicate the need for further study of the problem.

Data from the specimens examined indicate that the species is most commonly associated with deer-mice (*Peromyscus*), but it occurs on a wide variety of mammals and occasionally on birds. Adults are taken throughout the year, with seasonal maxima in spring and fall, but it is not known whether this is due to actual population differences.

LITERATURE CITED

CHAPIN, E. A. 1919 New species of North American Siphonaptera. Bull. Brooklyn Entom. Soc. **14** (2) : 49–62.

HOPKINS, G. H. E. 1954 The male and systematic position of *Epitedia testor* (Rothschild). The Entomologist **87**: 217–219.

HUBBARD, C. A. 1947 Fleas of western North America; their relation to public health. 533 pp. Iowa State College Press, Ames.

JORDAN, K. 1929 On a small collection of Siphonaptera from the Adirondacks, with a list of the species known from the state of New York. Novitat. Zool. **35**: 168–177.

MERRIAM, C. H. 1894 Laws of temperature control of the geographic distribution of terrestrial animals and plants. Nat. Geog. Mag. **6**: 229–238.

THE HELMINTH FAUNA OF NICARAGUA I. A NEW GENUS AND SPECIES OF CESTODE (*DILEPIDINAE*) FROM THE HUMMINGBIRD, *PHAEOCHROA CUVIERII ROBERTI*

KENNETH A. NEILAND

Department of Zoology, University of California, Los Angeles

The present paper is the first in a series which deal with helminths collected by the author in eastern Nicaragua during the months of June and July, 1954. Apparently, the helminth fauna of this region is practically unknown. To the best of the writer's knowledge only *Dicrocoelium biliophilus* (Price, 1928), taken from a Nicaraguan spider monkey which died in the National Zoological Park in 1926, has been previously reported (Price, 1928).

The collecting for this study was done at an agricultural experiment station located at El Recreo, Zelaya, Nicaragua, some 60 miles up the Rio Escondido from Bluefields, a south-eastern seaport. During this time, 193 amphibians, reptiles, fish, birds and mammals were examined for helminth parasites. Of these hosts, 167 were birds belonging to 84 genera and 35 families, many of which are restricted to the tropics. Forty-three percent of the species and 40 percent of the specimens of birds examined harbored approximately 50 species of trematodes, cestodes, nematodes and acanthocephalans in single or mixed infections. The group of helminths

Received for publication, May 25, 1955.

most commonly encountered were flukes belonging to the family *Dicrocoeliidae* Odhner, 1910. A study of this material should enhance our knowledge of the helminth fauna of Nicaragua and contribute to the zoogeography of avian helminths in Central America.

MATERIALS AND METHODS

The present paper is concerned with the description and taxonomic relationships of an unusual cestode recovered from the intestine of a hummingbird, *Phaeochroa cuvierii roberti*, for which the name *Arostellina reticulata* n. g., n. sp., is proposed. The author is aware of only one helminth previously reported from a trochiliform bird. Fuhrmann (1932) records *Anonchotaenia trochili* Fuhrmann, 1908, from *Eupetomena macrura* (Gm.) from eastern Brazil.

The details of the morphology were reconstructed from two gravid specimens stained in Kornhauser's hematein, cleared in cedar-wood oil and mounted whole. With this kind of preparation, the structural details could be readily observed, including many of the smaller collecting tubules leading from the testes to the vas deferens and the fine dorsal excretory canals. All measurements are given in millimeters; the drawings were prepared with the aid of a camera lucida.

I wish to acknowledge the aid of Dr. Thomas R. Howell of this department, who collected many, and identified all, of the birds examined. Without his generous help, the writer would have been unable to undertake and successfully complete even the limited amount of work carried out. Mr. John Zoeger also was of assistance in obtaining host animals. The collecting of the birds was supported in part by a grant from the University of California Association in Tropical Biogeography to Dr. Howell. Dr. Donald Heyneman, of this department, has read and constructively criticized this paper during its preparation.

Arostellina reticulata n. g., n. sp. (Figs. 1, 2)

Diagnosis: Arostellina: Strobila small, about 27 long with maximum width of 0.85 reached in incompletely gravid proglottids. Segments all longer than broad except early immatures. Scolex not well delimited from neck region. Suckers weakly developed, about 0.070–0.080 in diameter. Rostellum, rostellar sac and armature completely lacking. Excretory canals small, dorsal ones about 0.007 in diameter, ventral canals about twice as large. Transverse ventral communicating canal located near posterior margin of proglottid. Genital pores irregularly alternating and located in anterior fourth of segment at marginal prominence. Cirrus-sac small, about 0.11–0.13 long by 0.05 broad and not extending to excretory canals. Ductus ejaculatorius somewhat coiled within cirrus-pouch. Cirrus, when completely everted, about 0.09 long by 0.025 broad, aspinose. Vas deferens much coiled and about 0.015 in diameter. Genital ducts passing dorsal to excretory canals. Testes numerous, 60–76 in number, about 0.045–0.075 long by 0.040–0.075 broad and located posterior and lateral to ovary and vitelline gland. Ovary central, about 0.19–0.25 long by 0.18–0.19 broad and irregularly lobed along posterior margin. Vitelline gland about 0.060–0.090 long by 0.060–0.075 broad, anterior to ovary in region of ootype. Receptaculum seminis also anterior to ovary, 0.80–0.18 long by 0.04–0.12 broad, persisting in fully ripe segments. Vagina somewhat muscular in portion lateral to excretory canals, about 0.025 in diameter, with few coils between excretory canals and receptaculum. Uterus develops as reticulated network anterior to ovary and as filled with eggs, expanding to occupy entire proglottid. Eggs spherical, about 0.020–0.025 in diameter, not enclosed in capsules.

Host: Phaeochroa cuvierii roberti.

Habitat: Intestine.

Locality: El Recreo, 400 ft. elev., 10 miles west of Rama, Zelaya, Nicaragua.

Type: U. S. Nat. Mus. Helm. Coll. No. 37439.

Paratype: Helm. Coll., Dept. Zool., Univ. California, Los Angeles.

Arostellina n. g.

Diagnosis: Dilepididae: Small cestodes with weakly developed suckers. Rostellum and other scolex armature lacking. Genital pores irregularly alternate. Genital ducts passing dorsal to excretory canals. Testes numerous, posterior and lateral to central ovary. Vitelline gland and ootype anterior to ovary. Uterus reticulated, but not breaking up into egg capsules.

Type species: A. reticulata n. sp.

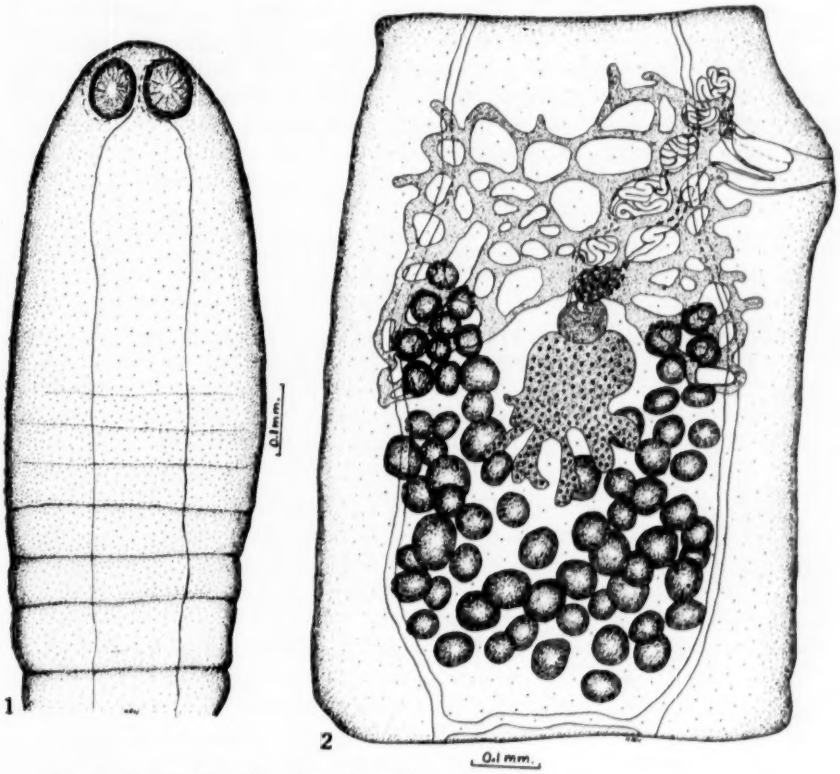


FIG. 1. Scolex and neck region, *Arostellina reticulata.*

FIG. 2. Ventral view of mature proglottid, *A. reticulata,* showing reproductive organs, with uterus at early stage of development.

The generic name is based on the absence of a rostellum, while the form of the uterus suggests the specific name.

The allocation of *Arostellina* n. g. to the *Dilepidinae* is consistent with the following features of its anatomy: numerous testes; no internal or external seminal vesicle; vas deferens greatly convoluted to form a storage organ; genital pores alternate; uterus reticulated, eggs not encapsulated, paruterine organ lacking. However, on the contrary, the author is not aware of any other dilepidine genus in which the ovary is posterior to the vitelline gland and ootype, an arrangement typical of the family *Tetrabothriidae* Linton, 1891. Either of the characters, absence of a rostellum or presence of a reticulated uterus, occurs only infrequently in the subfamily *Dilepidinae,* but the two characters until now have not been reported in combination. An example of the former is *Cotylorhipis* Blanchard, 1909,

also from a neotropical bird, but readily distinguished from the present form by the presence of sucker armature. *Cladogynia* Baer, 1937, *Pseudandrya* Fuhrmann, 1943, *Krimi* Burt, 1944, *Dendrouterina* Fuhrmann, 1912, *Similuncinus* Johnston, 1909, *Lapwingia* Singh, 1952, and *Dictymetra* Clark, 1952, all possess a reticulated uterus. An armed rostellum in these forms clearly separates them from *Arostellina*. Actually the anatomy of this genus presents a combination of characters which is not found in any family of cestodes known to the author. Although this might suggest the erection of a new supra-generic category or the emendation of one already in use for the reception of *Arostellina*, it is the writer's opinion that until additional closely related genera are found, it should be placed in the subfamily *Dilepidinae* of the family *Dilepididae*.

References Cited

Fuhrmann, O. 1932 Les ténias des oiseaux. Neuchâtel (Mém. Univ. Neuchâtel. **8**: 1–381).
Price, E. W. 1928 New helminth parasites from Central American mammals. Proc. U. S. Nat. Mus. Art. 4, **73**: 1–7.

EFFECTS OF CORTISONE ON *TRICHINELLA SPIRALIS* INFECTIONS IN NON-IMMUNIZED MICE[1]

Coit M. Coker[2]
Department of Parasitology, School of Public Health
University of North Carolina

This is a report on the first of a series of experiments testing the nature of the mechanism of acquired immunity of mice to *Trichinella spiralis*. Many prior investigators have demonstrated that mice develop acquired immunity to this parasite, as measured, for example, by adult or larval worm counts at specified time intervals following a challenging infection.

Taliaferro and Sarles (1939, 1942) showed that the immunity which develops in rats immunized against *Nippostrongylus muris* is due to the actions of antibodies supported by a cellular reaction against the worms. A similar mechanism has been suggested by Larsh (1953a) to explain the operation of acquired immunity against *Trichinella spiralis* in mice, namely, that it is due to the "primary actions of specific antibodies with secondary cellular cooperation." This hypothesis is based on a body of facts uncovered by various investigators (see review by Larsh, 1953b) which show that serological and cellular factors are intimately associated with the operation of the acquired immunity mechanism. Some of the most stimulating investigations on one or the other of these two factors in the immunity relations to *T. spiralis* were done by Mauss (1940), Oliver-Gonzalez (1940, 1941), Culbertson (1942) and Larsh and Race (1954).

To test this hypothesis, cortisone was selected as a tool likely to modify the

Received for publication, April 18, 1955.

[1] The work described in this paper was accomplished while the author was a graduate student in parasitology at the University of North Carolina School of Public Health. He is grateful to Dr. John E. Larsh, Jr., for his stimulating suggestions and for providing the opportunity and facilities for carrying out the experiment, and to Dr. W. W. Cort for aid in preparing the manuscript.

[2] Associate in Parasitology, Department of Microbiology, School of Medicine, University of Puerto Rico.

cellular phase of the immunity mechanism, for there are numerous reports in the literature verifying its suppressive action upon the lymphoid-macrophage system of a variety of animals, including mice. The current experiment was designed to determine the effects of cortisone upon the establishment and persistence of adult *T. spiralis* and upon the production of larvae in non-immunized mice. Although the experiment was performed with non-immunized mice, it will be evident that it deals with an acquired immunity resulting from the host's reaction to an initial infection. That such an acquired immunity can develop in non-immunized mice has been suggested earlier by Larsh (1953a) and Larsh and Race (1954). Other experiments in the series were performed on immunized mice and include a study of the effect of cortisone on the cellular reaction (Coker, 1954); their results will be published later.

MATERIALS AND METHODS

Mice used in the experiment were Swiss white mice of the strain maintained by the Parasitology Department of the University of North Carolina School of Public Health for a number of years. All were females, approximately 12 weeks old when they were given the worm infection. *Trichinella spiralis* larvae for infecting the mice were of the local strain which also has been maintained by the Department for several years, first in stock rats, later in stock mice. Stock cortisone was cortisone acetate Merck ("Cortone")* of a strength of 25 mg/ml in aqueous vehicle suspension. The stock suspension of cortisone was diluted, just prior to use, with sterile 0.85% sodium chloride solution to a strength of 10 mg/ml.

Procedures for obtaining larvae for infection, standardizing and delivering the infective doses, harvesting adult and larval worms from the infected animals, and making adult and larval worm counts were essentially the same as those described by Larsh and Kent (1949), with a few minor changes.

Cortisone was administered to the mice subcutaneously, in the region of the groin, using 0.5 mg. per dose given in 0.05 ml. A 0.25 ml. tuberculin syringe and a #24 needle were used. Alternate sides of an animal were injected from day to day. Injected control mice received 0.05 ml. doses of sterile 0.85% sodium chloride solution according to the same schedule as the cortisone experimentals.

Statistical analyses of the worm counts of the various groups were performed by means of the analysis of variance and of the "t"-test. A "t" value of 0.05 or less was accepted as significant.

Experimental design.—The main objective of the experiment was to determine if treatment of the host with cortisone would influence its normal defense against an infection with *T. spiralis*. The effect was measured by giving experimentally injected mice a standard infection with larvae, counting the numbers of worms persisting at various time intervals following the infection, counting the numbers of larvae produced by these worms, and comparing these figures with those of infected control mice not treated with cortisone. Saline-injected controls were added to determine if the injection treatment alone would influence the results.

Accordingly, 55 mice were divided into four major groups I–IV, each containing 15 animals, except group IV which contained ten (Table 1). Mice of all groups were each given an infection of 200 *T. spiralis* larvae. Each major group was sub-

* Supplied by Merck and Company, Rahway, N. J., through the courtesy of Dr. Ashton C. Cuckler.

divided into three subgroups (a), (b) and (c), of five mice each, which were necropsied for adult worm counts from both the small and large intestines at 14, 21 and 30 day intervals, respectively, following the infection. In addition, larval worm counts were performed on the mice of subgroups (c).

Group I mice served as uninjected controls, and group II mice as saline-injected controls. Group III mice received daily injections of cortisone beginning two days prior to infection and continuing to necropsy. Group IV mice received daily injections of cortisone beginning two days prior to infection and continuing to the 14th day following infection. It is apparent that group III(a) and group IV(a) were identical; hence, only one such subgroup was established.

The timing of the first necropsy, the selection of two schedules of administration of cortisone, and the counting of worms from both the small and large intestines of the mice were features of the design which were included in view of the finding by Larsh *et al.* (1952) that non-immunized mice show a significant loss of worms from the anterior small intestine between 11 and 14 days following infection. The implications of these aspects are clarified in the "Discussion" section of this report.

Larval worm counts were based on four mice per subgroup (c). Muscle tissue samples for histological study were taken from one mouse of each subgroup. Pathological findings from these samples are not presented at this time.

EXPERIMENTAL RESULTS

General results.—Average worm counts of the various groups and subgroups are presented in Table 1. Results of "t"-tests are shown in Table 2; in this table

TABLE 1.—*Average numbers of adult and larval* Trichinella spiralis *recovered from control and cortisone-treated mice at specified time intervals following a single infection with 200 larvae. Multiply larval counts by 100. There were five mice per subgroup, all females and 12 weeks old at the time of infection*

Groups		Adult worms			Larval worms
		(a) 14 days	(b) 21 days	(c) 30 days	30 days
I Uninjected controls (15 mice)	Small intestine	42.0	3.6	0.8	400.5
	Large intestine	34.2	19.8	0.8	
	Total	76.2	23.4	1.6	
II Saline-injected controls (15 mice)	Small intestine	25.6	3.4	0.4	438.1
	Large intestine	44.8	24.8	1.2	
	Total	70.4	28.2	1.6	
III Cortisone to necropsy (15 mice)	Small intestine	124.2	116.8	52.2	833.2
	Large intestine	0.8	1.2	1.2	
	Total	125.0	118.0	53.4	
IV Cortisone to day 14 (10 mice)	Small intestine	Same	122.4	45.2	825.9
	Large intestine	as	1.4	1.2	
	Total	III(a)	123.8	46.4	

the significance level values (0.01 and 0.02) indicate that the differences between the average worm counts of the groups compared are highly significant, since differences of such magnitude could be expected to occur by chance alone in only one or two cases, respectively, out of 100 similar samplings if the groups compared were not really different.

Adult worm counts.—Analyses of variance performed on the total adult worm counts of the subgroups indicated that there were no effects attributable to the trauma of injection or to the difference in schedule of administration of cortisone, *per se*. Therefore, the two control groups can be considered, where desired, as

actually one large control group, and the two cortisone groups as one large cortisone group. Selected "t"-tests (see first half of Table 2) indicated that 1) at 14 days more adult worms were recovered from the cortisone-treated animals (125.0) than from the controls (73.4); 2) the total adult worm counts of the two cortisone experimental groups at 14 and 21 days were essentially similar (125.0 and 120.0); and 3) the fairly large difference, at 14 days, between the counts of adult worms in the small intestine of the uninjected controls (42.0) and those in the saline-injected controls (25.6) was not significant.

At 14, 21 and 30 days following infection, the percentages of adult worms persisting from the infection of the control mice were 36.7, 12.9 and 0.8, respectively, as compared to 62.5, 60.4 and 24.9 for the cortisone-treated mice. In the control mice, appreciable numbers of adult worms were found in the large intestine, whereas in the cortisone-treated mice, the adult worms were found almost exclusively in the small intestine (Table 1).

TABLE 2.—*Selected counts of adult and larval worms compared for significant differences by means of the "t"-test*

Comparisons (Necropsy day indicated)	Type	Standard error $(\bar{X}_1 - \bar{X}_2)$	Degrees of freedom	Significance level
Controls (14) vs Cortisone (14)	Adult worm (total)	17.07	13	0.01
Cortisone (14) vs Cortisone (21)	Adult worm (total)	14.23	13	NS*
Uninjected controls (14) vs Saline controls (14)	Adult worm (sm. int.)	13.95	8	NS
Uninjected controls (30) vs Saline controls (30)	Larvae	192.0	3	NS
Continuous cortisone (30) vs 14-day cortisone (30)	Larvae	222.5	3	NS
Controls (30) vs Cortisone (30)	Larvae	133.7	7	0.02

* Not significant.

Larval worm counts.—Results of Student's "t"-test on the larval worm counts (see second half of Table 2) indicated that 1) there was no significant difference between the average counts of the uninjected controls (40,050) and the saline-injected controls (43,810); 2) there was no significant difference between the average counts of the two cortisone experimental groups (83,320 and 82,590); and 3) the average counts of the combined cortisone groups (82,950) were significantly higher than those of the combined control groups (41,930).

It was noticed, in making the larval worm counts, that fragments of worms were encountered in both the controls and the experimentals. They were probably due to young larvae which had arrived very recently in the musculature and were not sufficiently advanced in development to withstand the digestion-extraction process (McCoy, 1934). These could not be included in the counts. However, the fragments were far more abundant in the cortisone experimentals than in the controls.

DISCUSSION

Administration of cortisone, by either of the two schedules employed, to mice receiving an initial infection with *T. spiralis* resulted in longer persistence of more

adult worms in the intestine, and establishment of more larval worms in the musculature, than in control mice.

In the controls, the adult worms were found in both the small and large intestines (Table 1). They were lost rapidly from the small intestine, but some of these may have become re-established temporarily in the large intestine. In any event, the rate of loss from the large intestine also was quite rapid. In the cortisone-treated experimentals, on the contrary, the worms were found almost exclusively in the small intestine at all necropsy times. Although there was a significant loss of some of the worms of the cortisone groups between 21 and 30 days, it is likely that these passed out with the feces, without being forced to do so by the operation of a specific immunity mechanism, particularly in view of the fact that there is no evidence that they reestablished themselves in the large intestine.

Customarily it is found that when non-immunized mice receive an initial infection with *T. spiralis* larvae, a high percentage of the worms succeed in establishing themselves in the small intestine. This indicates that mice have little innate immunity against this parasite. However, a significant loss of adult worms from the anterior small intestine occurs between 11 and 14 days following infection (Larsh *et al.*, 1952). There is strong evidence that such a loss of worms in mice is due to the operation of a specific acquired immunity mechanism, rather than to either natural immunity or normal exhaustion of the worms in their reproductive and physiological cycle. Baughn (1952), using larval antigen, found a precipitin titer of 1 : 2 at seven days after infecting non-immunized mice. Larsh and Race (1954) discovered that just prior to the significant expulsion of adult worms from non-immunized mice there was a striking cellular reaction in the intestinal wall. On the contrary, in immunized mice the precipitin titer has been found to be much higher (Hendricks, 1950), the cellular reaction developed more rapidly (Larsh and Race, 1954), and the significant loss of worms occurred much earlier (Larsh *et al.*, 1952). In view of these observations the significant expulsion of worms from the small intestine of mice after one infection is comparable to the expulsion of worms in mice with acquired immunity, except that in the former the expulsion takes place later (Larsh, 1953a; Larsh and Race, 1954).

The results of this experiment strongly support the conception that the loss of worms in mice receiving an initial infection with *T. spiralis* is a manifestation of acquired immunity. The fact that significantly larger numbers of worms persisted in the cortisone-treated groups, and in particular the fact that these worms persisted chiefly in the small intestine, in direct contrast to the controls, is conclusive evidence that an immunity mechanism operated in the small intestine of the control mice and that the expression of this immunity mechanism was prevented in the experimental mice by treatment with cortisone. The loss of adult worms in the control mice, as opposed to the experimental mice, could not have been due primarily to senility of the worms unless it can be demonstrated that similar treatment of host mice with cortisone prevents senility of the worms. To ascribe it to natural immunity would be to ignore the demonstrated aspects of acquired immunity.

Counts of larval worms from the musculature of the cortisone-treated mice were almost twice as great as those from controls. These counts are not directly proportional to the numbers of adult worms persisting, but they indicate that the worms

in the cortisone-treated groups were well able to produce an abundance of larvae which later were capable of establishing themselves in the musculature.

In regard to the effects of cortisone given by the two different schedules, there were no significant differences in either adult worm or larval worm counts. The two schedules continued from an early time through the period, or through and beyond the period, respectively, when the immunity mechanism normally would come into play. This would seem to indicate that if the immunity mechanism is interfered with by cortisone during the period when it normally would develop, the mouse host is not able to generate a new mechanism in sufficient time to be effective within at least 30 days. Further experimentation, employing different schedules of administration of cortisone with reference to the times of infection and of necropsy, would be of value in clarifying this point.

The manner in which cortisone exerts the effects shown in these non-immunized mice is very likely due in large part to the suppression of the cellular response in the intestinal wall. The suppression of the cellular response by cortisone has been clearly demonstrated in immunized mice and shown to be associated with persistence of greater numbers of worms (Coker, 1954). Likewise, the results of the experiments of Weinstein (1955) with *Nippostrongylus muris* in cortisone-treated rats, and of Lindquist (1950) with *N. muris* in abnormal hosts, are recent indications that the cellular response plays an important role in the defense of the host against nematode parasites.

Stoner and Godwin (1953, 1954) found that cortisone treatment enhanced the susceptibility of non-immunized and of immunized mice given doses of *Trichinella* lethal to control mice. The present experiment (performed before their results appeared in press) provides a probable explanation for the enhanced susceptibility demonstrated by their work, in that it is likely that their cortisone-treated mice harbored more adult worms for a longer period, and, consequently, more larval worms, than their control mice.

Beneficial effects of ACTH and of cortisone therapy of human patients with clinical trichinosis have been reported (Rothenberg, 1951; Luongo *et al.*, 1951; Davis and Most, 1951; Rosen, 1952; Faiguenbaum, 1953; and various others). In nearly all cases there was marked clinical improvement during the therapy, the effect presumably being in the nature of an altered host sensitivity rather than effects upon the worms themselves. The results of the current experiments with mice seem to indicate that there are no adverse effects upon the worms due to cortisone treatment of the host. They suggest, moreover, that consideration should be given to the schedule of administration of cortisone to humans with trichinosis, for it is possible that if cortisone therapy were commenced too early, longer persistence of adult worms and larger larval worm burdens could result. This cannot be a definite statement, since the human host may react differently. Furthermore, cortisone treatment of the mice began before infection took place; in humans such a time relation would occur only accidentally. Finally, the size of the dose of cortisone may be an important factor.

SUMMARY

Two different schedules of cortisone treatment of non-immunized mice receiving an infection with *Trichinella spiralis* resulted in longer persistence of more adult

worms in the intestine, and establishment of more larval worms in the musculature, than in control mice.

Results of this experiment lend strong support to the view that non-immunized, mature mice develop a delayed measurable acquired immunity against an initial infection with *Trichinella spiralis*. They show that treatment of such mice with cortisone, beginning shortly prior to infection and extending through the period when this acquired immunity normally would manifest itself, prevented or greatly weakened the expression of the immunity.

References Cited

Baughn, C. O. 1952 The effect of adrenalectomy on natural and acquired resistance of mice to *Trichinella spiralis*. J. Elisha Mitchell Scient. Soc. **68**: 207–221.

Coker, C. M. 1954 Effects of cortisone on established immunity to *Trichinella spiralis* in mice, with reference to the role of the cellular factor(s) in the immunity mechanism. (Doctoral dissertation) University of North Carolina.

Culbertson, J. T. 1942 Active immunity in mice against *Trichinella spiralis* larvae. J. Parasit. **28**: 197–202.

Davis, W. M. and Most, H. 1951 Trichinosis; case report with observations of the effect of adrenocorticotropic hormone. Am. J. Med. **11**: 639–644.

Faiguenbaum, J. A. 1953 Triquinosis y cortisona. Bol. Inform. Parasit. Chilenas **8**: 9–11.

Hendricks, J. R. 1950 The relationship between precipitin titer and number of *Trichinella spiralis* in the intestinal tract of mice following test infections. J. Immunol. **64**: 173–177.

Larsh, J. E. 1953a Studies in old mice to test the hypotheses of local and general immunity to *Trichinella spiralis*. J. Infect. Dis. **93**: 282–293.

———1953b A review of recent immunological studies on trichiniasis in mice. Thapar Commemoration Volume, University of Lucknow, Lucknow, India, 159–172.

———, Gilchrist, H. B., and Greenberg, B. G. 1952 A study of the distribution and longevity of adult *Trichinella spiralis* in immunized and non-immunized mice. J. Elisha Mitchell Scient. Soc. **68**: 1–11.

——— and Kent, D. E. 1949 The effect of alcohol on natural and acquired immunity of mice to infection with *Trichinella spiralis*. J. Parasit. **35**: 45–53.

——— and Race, G. J. 1954 A histopathologic study of the anterior small intestine of immunized and non-immunized mice infected with *Trichinella spiralis*. J. Infect. Dis. **94**: 262–272.

Lindquist, W. D. 1950 Some abnormal host relationships of a rat nematode, *Nippostrongylus muris*. Am. J. Hyg. **52**: 22–41.

Luongo, M. A., Reid, D. H., and Weiss, W. W. 1951 The effect of ACTH in trichinosis—a clinical and experimental study. New England J. Med. **245**: 757–760.

Mauss, E. A. 1940 The *in vitro* effect of immune serum upon *Trichinella spiralis* larvae. Am. J. Hyg. **32–D**: 80–83.

McCoy, O. R. 1934 The effect of vitamin A deficiency on the resistance of rats to infection with *Trichinella spiralis*. Am. J. Hyg. **20**: 169–180.

Oliver-Gonzalez, J. 1940 The *in vitro* action of immune serum on the larvae and adults of *Trichinella spiralis*. J. Infect. Dis. **67**: 292–300.

———1941 The dual antibody basis of acquired immunity in trichinosis. J. Infect. Dis. **69**: 254–270.

Rosen, E. 1952 Cortisone treatment of trichinosis. Am. J. Med. Sc. **223**: 16–19.

Rothenberg, F. 1951 Treatment of trichinosis with cortisone. J. Med. Soc. New Jersey **48**: 517.

Stoner, R. D. and Godwin, J. T. 1953 The effects of ACTH and cortisone upon susceptibility to trichinosis in mice. Am. J. Path. **29**: 943–950.

——— and ———1954 The effects of adrenocorticotrophic hormone and cortisone upon acquired immunity to trichinosis in mice. Am. J. Path. **30**: 913–918.

Taliaferro, W. H. and Sarles, M. P. 1939 The cellular reactions in the skin, lungs and intestine of normal and immune rats after infection with *Nippostrongylus muris*. J. Infect. Dis. **64**: 157–192.

——— and ———1942 The histopathology of the skin, lungs and intestine of rats during passive immunity to *Nippostrongylus muris*. J. Infect. Dis. **71**: 69–82.

Weinstein, P. P. 1955 The effect of cortisone on the immune response of the white rat to *Nippostrongylus muris*. Am. J. Trop. Med. and Hyg. **4**: 61–74.

STUDIES ON *TRICHOSTRONGYLUS AXEI* (COBBOLD, 1879) I. SOME EXPERIMENTAL HOST RELATIONSHIPS*

J. H. Drudge, S. E. Leland, Jr., Zae N. Wyant, and G. W. Elam
Department of Animal Pathology
Kentucky Agricultural Experiment Station
Lexington, Kentucky

Natural infections of *Trichostrongylus axei* are recorded in a number of hosts including the ass, horse, mule, cattle, sheep, goat, several species of wild ruminants, pig and man (Mönnig, 1949, p. 203, and Nagaty, 1931). The scope of animals infected was extended by Nagaty (1931) in relegating the designation *T. extenuatus* (Railliet, 1898) from ruminants to synonomy with the previously described *T. axei* of equine hosts. Such a range and diversity of hosts is not only unusual for the genus, but also for nematodes in general and suggests the possible existence of biological strains. Only limited information is available relative to the transmission between the several hosts, although *T. axei* has been associated on a number of occasions with a severe gastritis in horses, cattle, and sheep. The transmission from cattle to sheep under natural conditions of exposure has been recorded (Stoll, 1936) on the basis of lesions observed in the abomasum of a necropsied lamb. Further, infections of *T. axei* of bovine origin have been established experimentally in lambs by Turner and Kates (1954) and Dikmans and Kates (1955). The reverse relationship of this parasite among these ruminant hosts also has been observed (Taylor, 1937, and Roberts, 1942) under natural conditions of exposure. Roberts, in addition, experimentally transmitted the infection developing in one of the calves to worm-free lambs. Britton (1939) reported on the experimental infection of a calf from a naturally infected horse, and a horse was utilized (Gibson, 1951) in isolating *T. axei* from a mixed infection of sheep nematodes by a single passage through the equine host. Other than the latter, the infection of the horse from the ruminant hosts has not been noted except for the finding by Foster (1937) of a higher infection rate in horses directly associated with cattle than in horses not under these conditions. As far as the present authors have been able to ascertain, the transmission of natural infections of *T. axei* in the horse to sheep has not been recorded.

Although *T. axei* displays a very wide host range under natural conditions, there appears to be no previously recorded attempt to explore the susceptibility of small laboratory animals to this form. In a review by Dikmans (1937) of the species of *Trichostrongylus* occurring in rodents and lagomorphs, *T. axei* was not listed. However, Baker (1933) has recorded the natural infection of a raccoon, and a recent report by Bull (1953) included the finding of a natural infection in a wild rabbit in New Zealand.

The present investigation was undertaken (1) to transmit experimentally *T. axei* between the horse, sheep, and cattle hosts, and (2) to test the infectivity of this form for small laboratory animals.

Received for publication, April 19, 1955.

* This investigation is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director of the Station and the Chief of the Animal Disease and Parasite Research Branch, U. S. Department of Agriculture. It is in cooperation with the Animal Disease and Parasite Research Branch, Agricultural Research Administration, U. S. Department of Agriculture.

MATERIALS AND METHODS

Five strains (three from horses, and one each from cattle and sheep) of *T. axei* occurring as natural infections were available for study. For the sake of clarity as to origin, the strains are given arbitrary letter designations in the following discussion. Infective larvae were obtained by culturing feces, employing mixtures of sphagnum moss or granular bone charcoal. The age of the infective larvae ranged from 7 to 14 days at the time of administration to the test animals, and the number of larvae in the test doses was estimated by dilution counts.

Several procedures were used to administer the infective larvae to the various hosts. For the horse, the larvae were mixed with oats in one instance, and in two instances the larvae were given via stomach tube. The calves were induced to nurse the larval suspension from a nippled pail. A dose syringe equipped with a Whitlock nozzle was used for the lambs. Miniature Whitlock-type nozzles, made from hypodermic needles and fitted to an ordinary glass hypodermic syringe, were employed on the small laboratory animals (rabbits, guinea pigs, hamsters, and rats).

All of the experimental animals were raised helminth-free, except for the horses. Before the latter were exposed, repeated flotations and cultures were made on the feces to obviate current *T. axei* infections.

Although fecal examinations for eggs and larvae were routinely made, the essential criterion to establish the maturation of the test exposures in the various hosts was the recovery and identification of the adult worms at necropsy. The recovery of this small form, intimately associated with the gastric mucosa, was facilitated by a preliminary digestion of the tissues with artificial gastric juice. The search for the worms was made routinely with the aid of a dissection microscope at a magnification of 13 times.

RESULTS

The various transmissions that have been completed with the five strains of *T. axei* are outlined in Figure 1. Of these strains, strain A from the horse has been studied most extensively.

In the trials with strain A in the ruminant hosts, direct infections were established in calf 41 and lambs 47 and 312.[1] Adult specimens of *T. axei* were recovered from the abomasums of these three animals when necropsied 42, 87, and 95 days, respectively, after exposure. Dosing calf 42 with infective larvae cultured from lamb 47 was followed by the isolation of adult *T. axei* from the abomasum 48 days later. The reciprocal of the latter experiment was also completed in the series of transfers stemming from the direct infection of calf 41. In this series, lamb 412 has not been necropsied, however, the infection was transmitted to a second lamb (42A) in which adult *T. axei* were readily demonstrated. From lamb 42A, the infection was returned to the horse by exposure of two weanlings (horses 2 and 3) via stomach tube. When horse 2 was sacrificed on the 56th day after exposure, numerous *T. axei* adults were found on the gastric mucosa. At the time of this writing, horse 3 has remained patent for 40 days after eggs were first observed on the 25th day.

From Figure 1, it will be noted that strain A has also been successfully estab-

[1] The authors are indebted to Dr. K. C. Kates of the Animal Disease and Parasite Research Branch, ARS, U.S.D.A., for verifying the identity of the specimens collected from lamb 312 and rabbit 1.

lished in several laboratory animals including the rabbit, guinea pig, and hamster. Rabbit 5 was infected directly from the horse, whereas the remainder of the small animal infections stemmed from the initial infection established in lamb 312.

In the studies with rabbits, only mature specimens of *T. axei* were observed in the worms collected from the stomachs of rabbits 1,[1] 4 and 5. These animals were examined post mortem at 39, 42, and 38 days, respectively, after exposure. The remaining rabbits in this series have not been necropsied at the time of this report. However, it is of interest to note that the infection in rabbit 8 has remained patent for 194 days after eggs were first detected on the 27th day following exposure. Rabbits 18 and 20 have remained patent for 147 and 81 days, respectively.

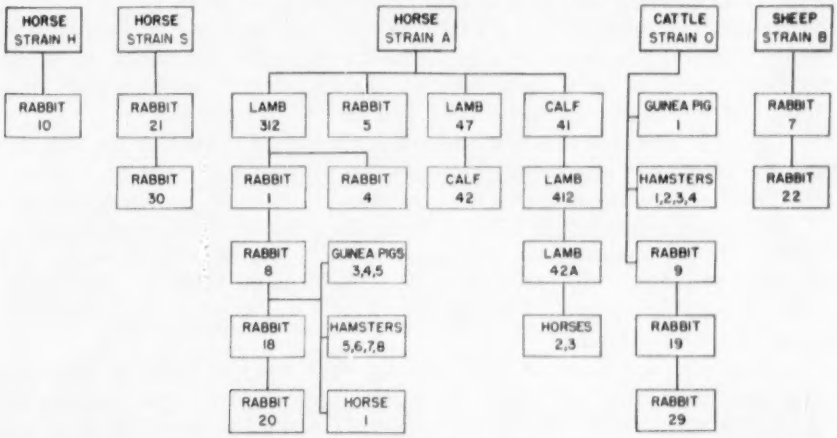


FIGURE 1. EXPERIMENTAL TRANSMISSIONS COMPLETED WITH TRICHOSTRONGYLUS AXEI.

Guinea pigs 3, 4 and 5 were given infective larvae cultured from rabbit 8. Although characteristic eggs were demonstrated on one or more occasions in the feces of all three animals, *T. axei* could be found in only one (5) at post-mortem examination on the 43rd day after the larvae were administered. Only mature forms were observed in this animal.

Hamsters 5, 6, 7, and 8 were also dosed with infective larvae from rabbit 8. With the exception of hamster 5 that received the lowest dose of infective larvae in the group, typical *T. axei* eggs were observed at least once in the feces of these animals between the 32nd and 60th days after exposure. Examinations by flotation on the 74th day were negative for eggs and the animals were sacrificed without making an examination for adult *T. axei*.

To complete the cycle in this series of transmissions with strain A, the infectivity for the horse of larvae cultured from rabbit 8 was tested. The animal (horse 1) for this trial was a yearling, on which repeated fecal flotation and culture examinations were consistently negative for *T. axei* before the test dose was given by mixing the larval suspension with oats. Periodic fecal examinations were continued until the 46th day after exposure, when the animal was sacrificed. On the 27th day, typical eggs were noted, otherwise the fecal examinations were uniformly negative for *T. axei*. However, the post-mortem examination of the gastric mucosa revealed

the presence of a light infection of *T. axei* adults including egg-bearing females.

Investigation of two additional strains of *T. axei* from horses has been limited to a corroboration of the experience in the rabbit host with strain A. In a single trial with strain H, infective larvae matured in a rabbit (10), and adult worms were isolated from the stomach on the 70th day after dosing. Similarly, the exposure of a rabbit (21) to larvae cultured from a third naturally infected horse (strain S) resulted in the maturation and the subsequent transmission to a second rabbit (30). One-hundred days after exposure, rabbit 21 was necropsied, and adult *T. axei* were found on the gastric mucosa.

To enlarge upon the foregoing findings in the small laboratory animals, additional trials were conducted with these animals as hosts for a calf strain (O) of *T. axei*. This strain was obtained from a field case exhibiting symptoms of parasitism in which *T. axei* eggs appeared in a fecal examination as the predominant nematode involved. Infective larvae were cultured and administered simultaneously to a rabbit (9), guinea pig (1), hamsters (1, 2, 3, and 4), and two rats.

Typical *T. axei* eggs were first observed in the feces of rabbit 9 on the 25th day and this animal was subsequently necropsied on the 84th day. Mature specimens of *T. axei* were readily recovered from the stomach; however, an intensive search of the stomach and intestines failed to reveal the presence of any other nematode species. In the meantime, the infection in rabbit 9 was transmitted in sequence to rabbits 19 and 29. Necropsy findings on rabbit 19 on the 125th day after dosing, were the same as for rabbit 9, in that only *T. axei* was found in the examination of the gastro-intestinal tract.

Guinea pig 1 was sacrificed on the 29th day which was four days after eggs were first seen in the feces. Although egg-bearing female *T. axei* were found on the gastric mucosa, the bulk of the females recovered contained no eggs. All the worms from this animal were fifth stage forms, but both sexes were characterized by their small size. No other species of nematode was found in this animal.

Strain O larvae also matured in three of four golden hamsters. The exception occurred in hamster 1 that died of an undetermined cause on the fourth day after dosing. Present in the stomach was a large number of larval forms that had grown 1.5 to 2 times longer than the infective third stage larvae of *T. axei*. Hamster 2 was necropsied on the day (29th) following the first observation of eggs in the feces. In addition to adult *T. axei*, both early and late fourth stage larval forms were encountered in the stomach. Early fourth stage larval forms were also found in the small intestine. Only adult *T. axei* were collected from the stomachs of hamsters 3 and 4 which were sacrificed on the 35th day. The worms from the latter two animals were in general smaller than the adult forms encountered in the ruminant, horse, and rabbit trials.

The observations on the rats produced no evidence of the development of the test doses of strain O infective larvae. Negative findings attended repeated fecal flotations that were made through the 38th day after exposure. Both rats were examined post-mortem on the following day, but no worms were recovered in an intensive search of the gastro-intestinal tract.

A sheep strain (B) of *T. axei* also matured in the rabbit. The origin of this strain was a local flock naturally infected with a mixture of nematodes in which the presence of *T. axei* was indicated by fecal examination. Rabbit 7 became patent

after being dosed with infective larvae and showed a continued patency for a period of five and one-half months, or until necropsy. Only adult *T. axei* were present in the stomach, and a thorough search of the intestines failed to demonstrate the presence of another nematode species. During the seventh week of patency, larvae were cultured from rabbit 7 and given to a second rabbit (22). The latter's feces contained eggs on the 28th day and mature *T. axei* were found on the gastric mucosa at postmortem examination on the 104th day. In contrast with the findings on rabbit 7, *T. colubriformis* adults were recovered from the small intestine of rabbit 22.

DISCUSSION

The trials in the ruminant hosts demonstrate the infectivity of a strain of *T. axei*, originally encountered as a natural infection in the horse, for sheep and cattle, both directly and indirectly. The infection of a calf directly from the horse corroborates the earlier observation by Britton (1939) on this point. The infection of lambs directly from the horse is believed to be the first record of this relationship with an equine strain of *T. axei* although Gibson (1951) was successful in transmitting an ovine strain in sequence from sheep to horse to sheep. Furthermore, the experimental cross-transmission between the lambs and calves are in accord with the previous observations of Roberts (1942), Stoll (1936), Taylor (1937), Turner and Kates (1954) and Dikmans and Kates (1955). In the present study, the return of the infection to the horse after passage through a calf and two lambs indicates that the infectivity for the species of origin is retained, which corroborates and extends the observations of Roberts (1942) and Gibson (1951) on the single passage of ovine strains through a calf and a horse, respectively. The remaining transfer with this strain was not completed, but it seems reasonable that infective larvae cultured from the calves would have matured in the horse. In this phase of work, the number of animals involved was too small to permit an evaluation of these species as hosts for this strain of *T. axei*; however, studies are underway relative to the pathogenicity for sheep and cattle. Although both sheep and cattle were successfully infected with this strain, the observations do not preclude the existence of strains of *T. axei* exhibiting preferences among these hosts.

From the present investigations, compatibility between *T. axei* and the rabbit was evident in several respects. Experimental exposures with larvae from five different sources including the equine, bovine, and ovine hosts matured regularly in each of the animals. Although the range (200–10,000) of infective larvae in the test exposures was restricted, no untoward effect was observed in any of the animals. Finally, the infections established with the indicated range of doses of larvae have been observed to remain patent for more than six months. More detailed investigations on the quantitative characteristics of *T. axei* infection in the rabbit are in progress and will be reported at a later date.

The regularity in which the rabbits were infected directly from the equine, bovine, and ovine hosts raises the question as to why *T. axei* has been reported in wild rabbits only once previously (Bull, 1953). Under natural conditions, it would seem that the associations on the range and the dietary habits of rabbits and the above domestic animals would provide for the cross-transmission of this form. Thus, the explanation may be that the presence of *T. axei* has been overlooked. On this point, the combination of its small size and intimate association with the

gastric mucosa made the detection rather difficult in the present study, even though its presence was practically assured and a specific search was made. Further investigation, in the nature of a survey designed to detect *T. axei* in rabbits, would not only answer the question posed above but would also serve to clarify the role rabbits play in the natural transmission of *T. axei* between its domestic animal hosts.

Although *T. axei* from the horse and calf matured in the hamster, the limited observations indicated that the development in this experimental host was more retarded than in the rabbit. This was evident in the recovery of fourth stage larvae along with adult forms on the 29th day after exposure in one animal, and the generally small size of the adult worms found in two animals on the 35th day after infective larvae were administered. The possibility of re-infection in these animals is discounted because of insufficient time for larval development between first patency and necropsy of the first animal and because only adult forms were recovered from the latter two animals.

Restricted development of *T. axei* was also indicated in the guinea pig. This was evident in the smallness of the adult worms encountered in one animal necropsied 29 days following larval administration. In another trial, mature *T. axei* was demonstrated in only one of three guinea pigs simultaneously exposed and later necropsied on the 43rd day, although characteristic eggs were observed on one or more occasions in the feces of all three. This suggests that the infection in the guinea pig may be more transient.

Experimental infections of *T. axei* in the rabbit, guinea pig, and hamster present new host-parasite relationships that may be utilized in preliminary investigations of various aspects of trichostrongyle infections of domestic animals. The advantages, both technical and economic, presented by a recognized domestic animal pathogen established in a small laboratory animal are obvious, while the types of investigation suggested (Baker, 1954) recently for the intestinal trichostrongyloid (*Nematospiroides dubius*, Baylis, 1926) of the mouse would also appear appropriate for *T. axei* in a laboratory host.

Establishing in a rabbit an experimental infection of *Trichostrongylus colubriformis* derived from the sheep host is in accord with previous reports (Dikmans, 1937) of natural infections in wild rabbits. The failure to recover this species from the rabbit exposed directly from the sheep was probably due to the delay in sacrificing the animal, during which the infection was spontaneously eliminated. This parasite was encountered as an incidental finding to the main line of the investigation, however, the potential usefulness in research of this host-parasite relationship also appears to have received little attention. The common occurrence of *T. colubriformis* in ruminants presents a problem in obtaining *T. axei* in pure culture. Employing the horse as a source of *T. axei*, obviates this problem because *T. colubriformis* is not known to occur in the horse.

SUMMARY

A natural infection of *T. axei* in a horse was experimentally transmitted to lambs and calves both directly and indirectly. In sequence, following the infection of a calf and two lambs, this strain of *T. axei* again matured in the horse. Domestic rabbits were successfully infected with five strains (three equine, one ovine, and one bovine) of *T. axei* initially encountered as natural infections in these hosts. Exposures with the bovine and one of the equine strains also developed to maturity

in both guinea pigs and hamsters, although the limited experience suggested that these animals were not as suitable hosts as the rabbit. No evidence of maturation was found in rats exposed to the bovine strain larvae. *T. colubriformis*, which accompanied the ovine strain of *T. axei*, also matured in rabbits.

REFERENCES

BAKER, D. W. 1933 Trichostrongylus in New York State. Cornell Vet. **23:** 61–73.

BRITTON, J. W. 1939 Trichostrongylosis in equines. Cornell Vet. **29:** 322–330.

BULL, P. C. 1953 Parasites of the wild rabbit, *Oryctolagus cuniculus* (L.) in New Zealand. N. Zealand J. Sc. and Technol., Sect. B, **34:** 341–372.

DIKMANS, G. 1937 A note on the nematode genus *Trichostrongylus* occurring in rodents and lagomorphs, with descriptions of two new species. J. Wash. Acad. Sc. **27:** 203–209.

DIKMANS, G. AND KATES, K. C. 1955 Trichostrongylosis in cattle. Proc. Helminth. Soc. Wash. **22:** 42–46.

FOSTER, A .O. 1937 The occurrence of *Trichostrongylus axei* (Cobbold) in equines in Panama. J. Parasit. **23:** 573–574.

GIBSON, T. E. 1951 The pathogenesis of *Trichostrongylus axei* in sheep. State Vet. J., Ministry of Agriculture, Great Britain, 6 pp.

MÖNNIG, H. O. 1949 Veterinary Helminthology and Entomology, 3rd Edition, 1949 Printing, Bailliere, Tindall, and Cox, London, 427 pp.

NAGATY, H. F. 1931 On the identity of *Trichostrongylus axei* (Cobbold, 1879) Railliet and Henry, 1909, and *T. extenuatus* (Railliet, 1898) Ransom, 1907. Ann. Trop. Med. and Parasitol. **25:** 107–121.

ROBERTS, F. H. S. 1942 The host specificity of sheep and cattle helminths, with particular reference to the use of cattle in cleansing sheep pastures. Austral. Vet. J. **18:** 19–27.

STOLL, N. R. 1936 Observations on cattle nematode infections with a demonstration of their secondary transmission to grazing sheep. J. Parasit. **22:** 386–407.

TAYLOR, E. L. 1937 Parasitic gastritis. The transference of the causative helminths from sheep to cattle. Vet. J. **93:** 353–355.

TURNER, J. H. AND KATES, K. C. 1954 The pathogenic effects on lambs of a bovine strain of the stomach hairworm, *Trichostrongylus axei*. J. Parasit. **40** (5 sec. 2) : 13.

MYXOSPORIDIA IN FISH IN WATERS EMPTYING INTO GULF OF MEXICO

R. H. RIGDON AND JOHN W. HENDRICKS

Laboratory of Experimental Pathology
University of Texas Medical Branch
Galveston, Texas

MYXOSPORIDIA are commonly found in a variety of fish widely distributed throughout the world. Sprague (1954) recently stated "The writer knows of no myxosporidia which have been reported from the Gulf of Mexico, although there is no reason to doubt that they occur there." In the spring of 1954 three small fish were caught in a slough and a lagoon on opposite sides of the Galveston Ship Channel only a short distance before the Channel waters enter the Gulf of Mexico. MYXOSPORIDIA were present in each of the fish. These observations are recorded now to show that MYXOSPORIDIA have been demonstrated in fish in the Gulf waters.

MATERIALS AND METHODS

The three fish were caught with a minnow net on three occasions between May 20th and June 19th, 1954. Within 24 hours after being caught each was brought

Received for publication, March 14, 1955.

alive into the laboratory. They were sacrificed immediately by placing them in a 10 percent solution of formaldehyde. Several sections were made including the tumor and the ulcers with cross-sections of the body. These were also fixed in the formaldehyde solution. Paraffin sections were prepared and stained by the following techniques: hematoxylin and eosin, Masson's trichrome stain, Mallory's aniline blue stain and Wilder's reticulum stain.

The lagoon where two of these fish (1 and 2) were caught was parallel to the Gulf of Mexico and was located approximately one-half mile from the beach. It was approximately 5,000 feet long and 400 feet wide. The water communicated with the Galveston Ship Channel through the South Jetties. Fish 3 was caught in a small slough near the North Jetties approximately one-fourth of a mile from the Gulf of Mexico. Water from the Gulf enters this slough with high tide. There is also a connection between this slough and the East Galveston Bay. The latter is part of the Ship Channel.

OBSERVATIONS ON FISH

Fish 1. This fish, a sheepshead minnow (*Cyprinodon variegatus Lacepede*), measured 5 by 1.8 cms. (Pl. I, fig. A). Approximately one dozen tumors protruded from the surface of the body. A majority of these tumors were definitely circumscribed while a few were continuous one with another. The external surface was smooth; some were gray-white; others were yellowish-gray and black. The tumors appeared to be continuous with the muscle. The color apparently was determined by the anatomic location on the fish, being due to the variations in pigmentation of different parts of the body. No ulcerations were present on the body of this fish.

Histologically these tumors represented only masses of spores located in the muscle. Sometimes the spores extended in an irregular pattern down into the muscle (Pl. I, figs. B and C) while other groups of spores were more or less sharply demarcated by a capsule-like structure from the surrounding muscle. A similar capsule frequently was present within the mass of spores giving the tumor a lobulated appearance. There was no inflammatory reaction about any of these groups of spores. All the spores appeared alike. Their microscopic characteristics are shown in Pl. I, figs. D, E and F.

The second fish, a black chub (*Fundulus similis*), (Pl. II, fig. A) measured 8 by 1.5 cms. A superficial ulcer, 17 by 12 by 2 mm., was located on the dorsum just posterior to the head. The muscle in this area was necrotic and many bacteria were present. There was a minimal amount of cellular reaction about the periphery of this necrotic lesion. This reaction was more pronounced immediately beneath the skin. Small groups of spores were present within some of the muscle cells (Pl. II, figs. B and C) while other groups of spores were located between the muscle cells. There seemed to be fewer spores present in this fish than in the other two fish examined. Acute degeneration was conspicuous in many of the muscle cells (Pl. II, fig. B). There was no inflammatory reaction about any of the groups of spores. The spores in fish 2 (Pl. II, figs. D and E) were much smaller than those in fish 1; however, their shape was similar.

The third fish, a mullet (most like *Mugil cephalus*), measured 13 by 2.5 cms. There were three superficial ulcerated areas approximately 1.0 cm. in diameter and 2 mm. in depth. These were located over the dorsum of the head and one was

posterior to the gills on each side of the body. Strands of green algae seemed to be growing from these three ulcerated lesions. There were many large groups of spores located between the muscle cells (Pl. III, fig. A). Some groups of spores were present in the interstitial tissue adjacent to small blood vessels. Only one group of spores was found with a capsule surrounding the organisms such as was so frequently observed in fish number 1. Acute degeneration was present in the muscle cells in the area of the spores (Pl. III, fig. A) identical with the necrosis that occurred in fish number 2. No cellular reaction was present about any of these spores.

The spores in fish 3 (Pl. III, figs. B, C, D, E and F) were entirely different from those in either fish 1 or fish 2. The spores in this fish seemed to have four polar capsules. A small nodule was present on one end of many of these spores as shown in Pl. III, figs. C, E and F and also in the drawing (Pl. III, fig. B). It was most difficult to photograph the spores in fish 3 although the sections were prepared the same as those for fish 1 and 2.

DISCUSSION

The attempt to identify the PROTOZOA in these three fish was unsatisfactory since all the material had been fixed in formaldehyde. There can be no doubt that these spores represent MYXOSPORIDIA. Those in fish 1 probably represent a species of *Myxobolus* and those in fish 2 may be species of *Myxosoma* while those in fish 3 may be a species of *Kudoa*. It is interesting, however, to note that the histologic appearance of the degenerated muscle is similar and, furthermore, both fish 2 and 3 had specific (superficial) ulcers on the body. Some of the spores in fish 2 were limited to a specific muscle cell (Pl. II, fig. B) although others were located between the muscle cells in the same position as those found in fish 3. Attempts have been made on several occasions without success to obtain infected fish from the same lagoon and slough so special techniques could be carried out to identify the organisms. During past years fish with ulcerations and algae growing from the lesions have been observed about the Jetties by one of us (J.W.H.).

The spores in each of these three fish were restricted to the muscle, but whether the spores originally were restricted to the muscle fibers cannot be determined from these histologic studies. Only fish 2 showed spores at this time definitely restricted to the muscle cell. Histologic sections on these three fish did include portions of the viscera but no organisms were found other than in the muscle. It therefore would seem that these MYXOSPORIDIA grow primarily in the muscle. Davis (1924) in a study of "wormy" halibut stated "only a very few species (myxosporidia) ever enter the muscle fibers, and even in these forms the principal seat of infection is the connective tissue. The present species appears, therefore, to be unique among the myxosporidia, since so far as known it develops only within the muscle fibers and never in the surrounding tissue."

No inflammatory reaction was present about the different groups of spores. The ulcers in fish 2 and 3 may have resulted from the MYXOSPORIDIA, however, from this pathologic study it cannot be proved. These ulcers did contain bacteria but again one cannot say whether these bacteria were the cause of the ulceration. There was some inflammatory reaction associated with the necrosis; however, the extent of the inflammatory reaction was not proportional in any way to the extent

of the necrosis. The extensive acute degeneration of the muscle cells in the area where the groups of spores were found would suggest that this degeneration may have resulted from some "toxic" substance associated with the MYXOSPORIDIA as has been suggested by Nigrelli (1953). Thompson (1916) described the gross appearance of muscle of "wormy" fish but made no histologic observations.

The multiple lesions in fish 1 are interesting since they originally were thought to be new growths and were described as tumors. Similar macroscopic lesions produced by some genera of MYXOSPORIDIA have been referred to as myxosporidian cysts by Kudo (1946, p. 517). Nigrelli (1953) recently stated that "tissue growths associated with myxosporidia are most striking, sometimes presenting tumors that are difficult to distinguish from true neoplasia." Several observations on cystic tumors produced by MYXOSPORIDIA were cited by Nigrelli (1953) in which the overlying epithelium was hypertrophied and in one case it was either papillary or warty in appearance. He (1948) suggested that intracellular and intercellular MYXOSPORIDIA elaborate enzymes (hyaluronidase) or other chemical sustances that may be the stimuli responsible for cellular hypertrophy, hyperplasia and even metaplasia which is often noted in these infections. The histologic observations as made on the three fish reported at this time do not show the cellular hypertrophy or cell enlargement that Nigrelli (1953) found "to be a characterisic reaction in fishes to intracellular and intercellular myxosporidians."

SUMMARY

Three fish caught in a lagoon and in a slough, the water of which is continuous with that of the Gulf of Mexico, were infected with MYXOSPORIDIA. The genus could not be determined satisfactorily since all the material was fixed in formaldehyde. Apparently this is the first report of MYXOSPORIDIA in the waters of the Gulf of Mexico.

ACKNOWLEDGMENT

We wish to acknowledge the assistance given to us in this study by many individuals throughout this country. Especially do we appreciate the help given by Drs. J. G. Mackin, R. R. Kudo, Asa C. Chandler and our associates at the Medical Branch. Dr. Henry Hildebrand kindly identified the fish.

REFERENCES CITED

DAVIS, H. S. 1924 A new myxosporidian parasite, the cause of "wormy" halibut. Bureau of Fisheries, Document 957, pp. 1–5.

KUDO, R. R. 1946 Protozoology. Charles C Thomas, Springfield, Ill., 3rd Ed., 778 pp.

NIGRELLI, R. F. 1948 Prickle cell hyperplasia in the snout of the redhorse sucker (*Moxostoma aureolum*) associated with an infection by the myxosporidian *Myxobolus moxostomi*. Sp. Nov., Zoologica **33**: 43–46.

———1953 Tumors and other atypical cell growths in temperate freshwater fish of North America. Trans. Am. Fisheries Soc. 1953. 83rd Annual Meeting, Sept. 16–18, pp. 262–296.

SPRAGUE, V. 1949 Protozoa. In: Gulf of Mexico—its origin, waters and marine life. Paul S. Galtsoff, Ed. U. S. Fish and Wildlife Service, Fish. Bull. **55** (89): 243–256.

THOMPSON, W. F. 1916 A note on a sporozoan parasite of the halibut. Rep. Comm. of Fish., Prov. Brit. Columbia, pp. 127–129.

Explanation of Figures

Plate I

Fig. A. A sheepshead minnow with tumors on one side of the body. Similar lesions were present on the opposite side.

Fig. B. Myxosporidia spores located primarily in the muscle were the basis for these tumors. The spores infiltrated down between some of the muscle cells. H & E × 110.

Fig. C. The groups of spores sometimes appeared lobulated as a result of a membrane separating masses of the spores. H & E × 675.

Fig. D. A drawing of the spores.

Figs E. & F. The spores always occurred in pairs with a capsule surrounding the group. Wilder's reticulum stain × 1900.

Plate II

Fig. A. A black chub with a large superficial ulcer on the dorsum of the body.

Fig. B. Groups of the spores were restricted to individual muscle cells. Note the extensive degenerative changes in the muscle cells located around the organisms. H & E × 140.

Fig. C. The spores are more or less uniform in size in this fish. H & E × 602.

Figs. D. & E. The spores occur in pairs. They are smaller and their capsule also is thinner than that of the spores observed in fish 1. Wilder's reticulum stain × 1900.

Plate III

Fig. A. The spores in this fish (a mullet) were located between the muscle cells. Note the degeneration of the muscle cells. H & E × 602.

Fig. B. Drawing of the spores.

Fig. C. One pair of spores is pear-shaped. This is an infrequent finding in this fish. Wilder's reticulum stain × 1900.

Figs. D. & E. Spores with four polar capsules are typical of this infection. Wilder's reticulum stain × 1900.

Fig. F. The nodules so frequently present at the end of the spores in this fish are nicely shown in this illustration. Wilder's reticulum stain × 1900.

PLATE I

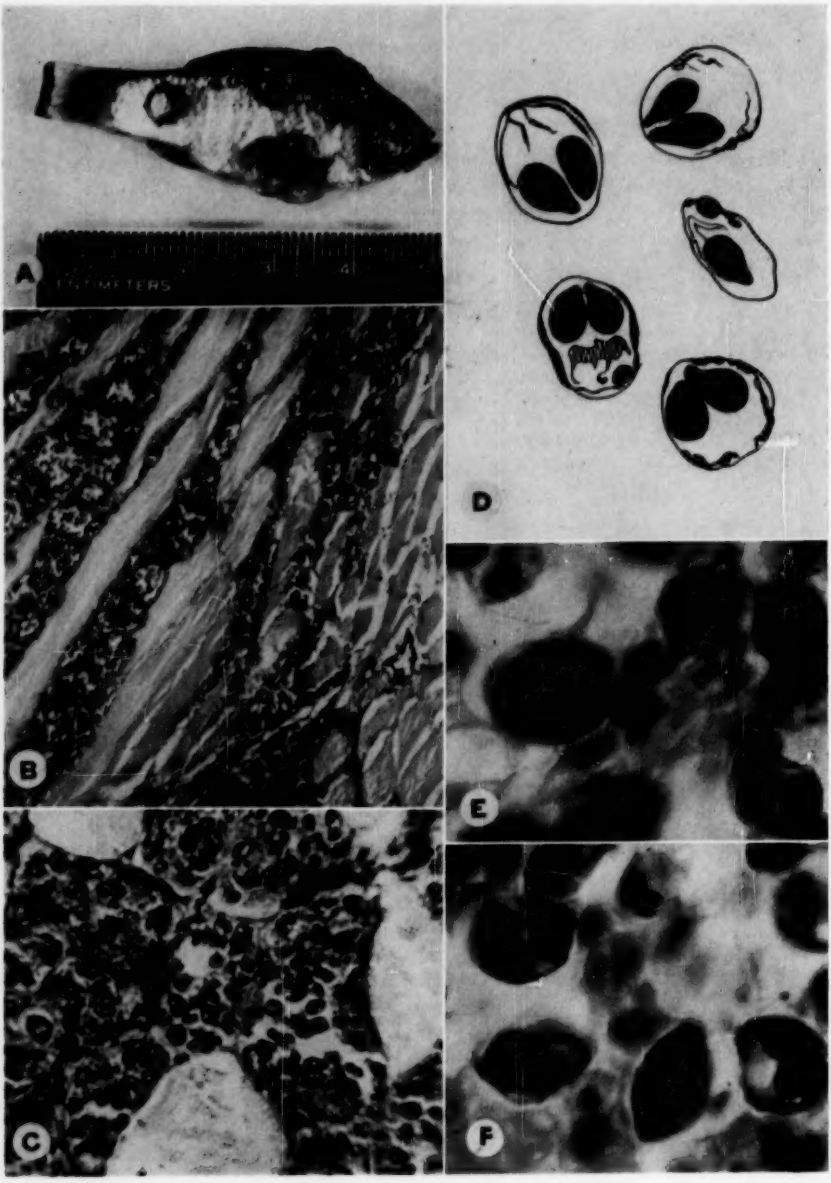


PLATE II

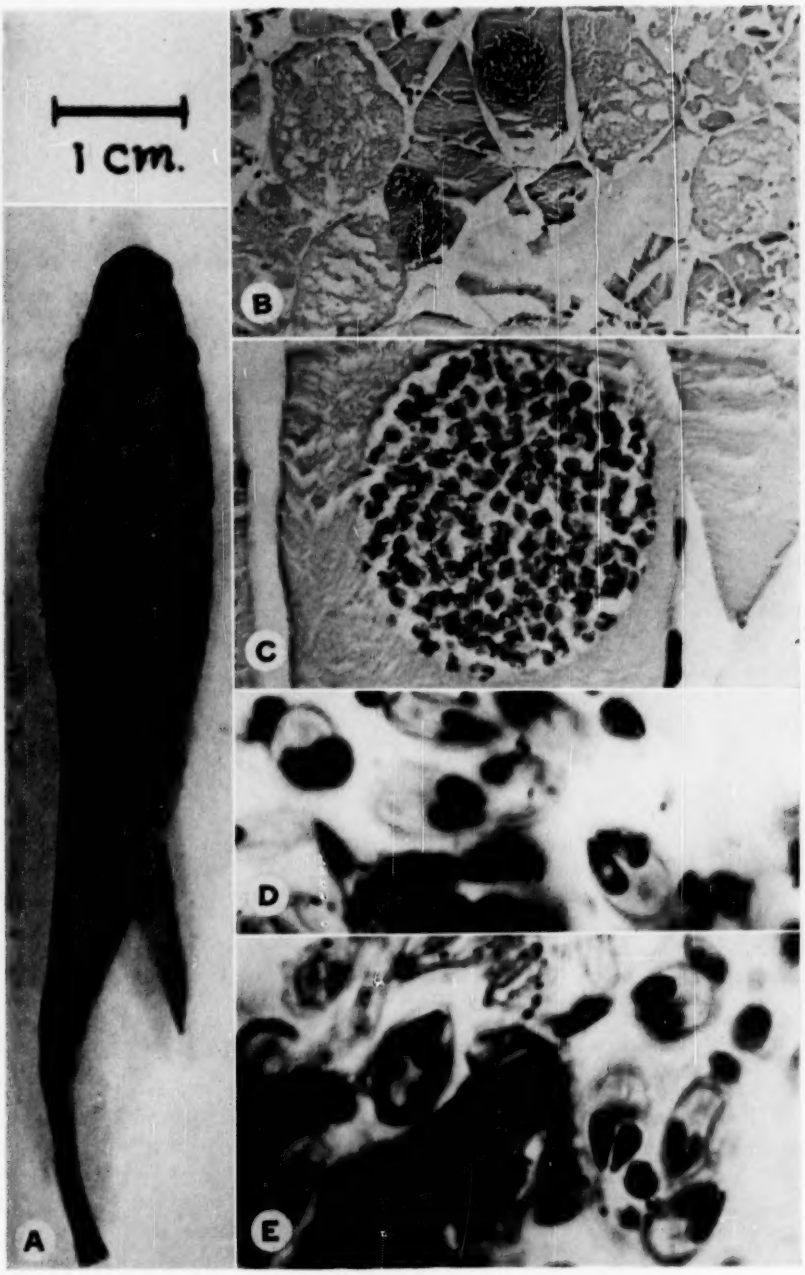
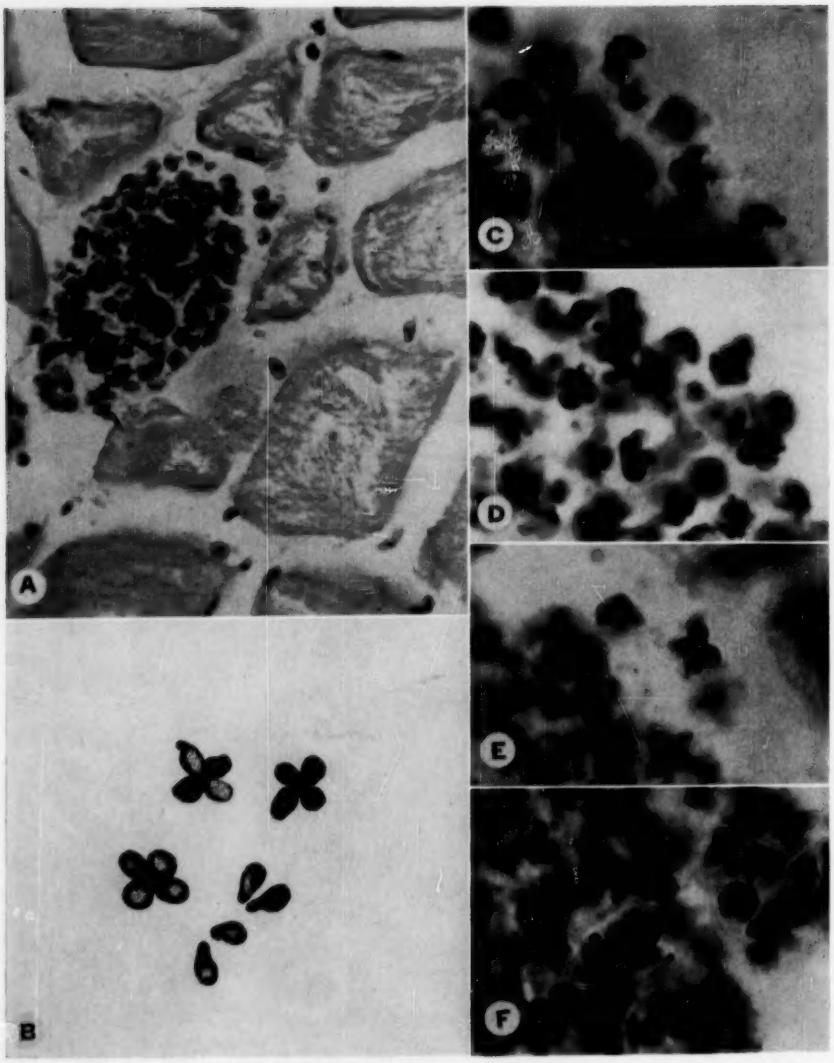


PLATE III



STEPTOLAELAPS (ACARINA: LAELAPTIDAE) A NEW GENUS OF MITES PARASITIC ON NEOTROPICAL RODENTS

DEANE P. FURMAN
Department of Entomology and Parasitology
University of California
Berkeley

Among the collections made by the author in 1953 as part of a study of the parasitic mites of Mexico were many robust, active acarines from pocket mice, *Liomys* spp. These have been identified as *Laelaps liomydis* Grant. Through the courtesy of Lieutenant Colonel Robert Traub a collection of parasitic mites from Venezuela was made available for study and comparison with the Mexican fauna. Among these were numerous specimens from *Heteromys* and other rodents which represent a new species of mite closely related to *L. liomydis*.

Study of these forms was facilitated by the loan of the allotype male and a female specimen of *L. liomydis* by C. Donald Grant, and the loan of a cotype of *Liponysella madagascariensis* (Hirst) by the British Museum of Natural History. Identifications of Mexican mammals were made by Drs. Bernardo Villa R. and Seth Benson. Dr. E. W. Jameson, Jr. kindly offered helpful comments on the manuscript.

It is the author's belief that *L. liomydis* and the new species described here represent a new genus. Grant (*in litt.*) agrees with this interpretation.

Steptolaelaps new genus

Robust spiny mites with characteristics of subfamily LAELAPTINAE as given by Vitzthum (1942, pp. 768–769). Chelicerae of both sexes with movable digit toothed and larger than the simple, lobe-like, fixed digit; from one to several long pseudo-setae arising around base of movable digit. Hypopharynx a simple, elongate lobe, narrowly rounded apically and provided with numerous microchetae. Coxae of both sexes with some ventral setae enlarged as stout, blunt spines. Coxae II without antero-dorsal spur. Trochanter and femur of leg I each with a thickened, short, ventral spine. Peritreme narrowly elongate. Female with three pairs of setae on genito-ventral plate; anal plate elongate, bearing a pair of adanal setae and a larger postanal seta. Holoventral plate of male entire. Tectum of both sexes an indistinct convex lobe located dorsally at the base of the gnathosoma. Dorsal plate with marked shoulders anteriorly, acuminate posteriorly, leaving much of opisthosoma uncovered. Dorsal plate and uncovered dorsal cuticula bearing numerous strong setae.

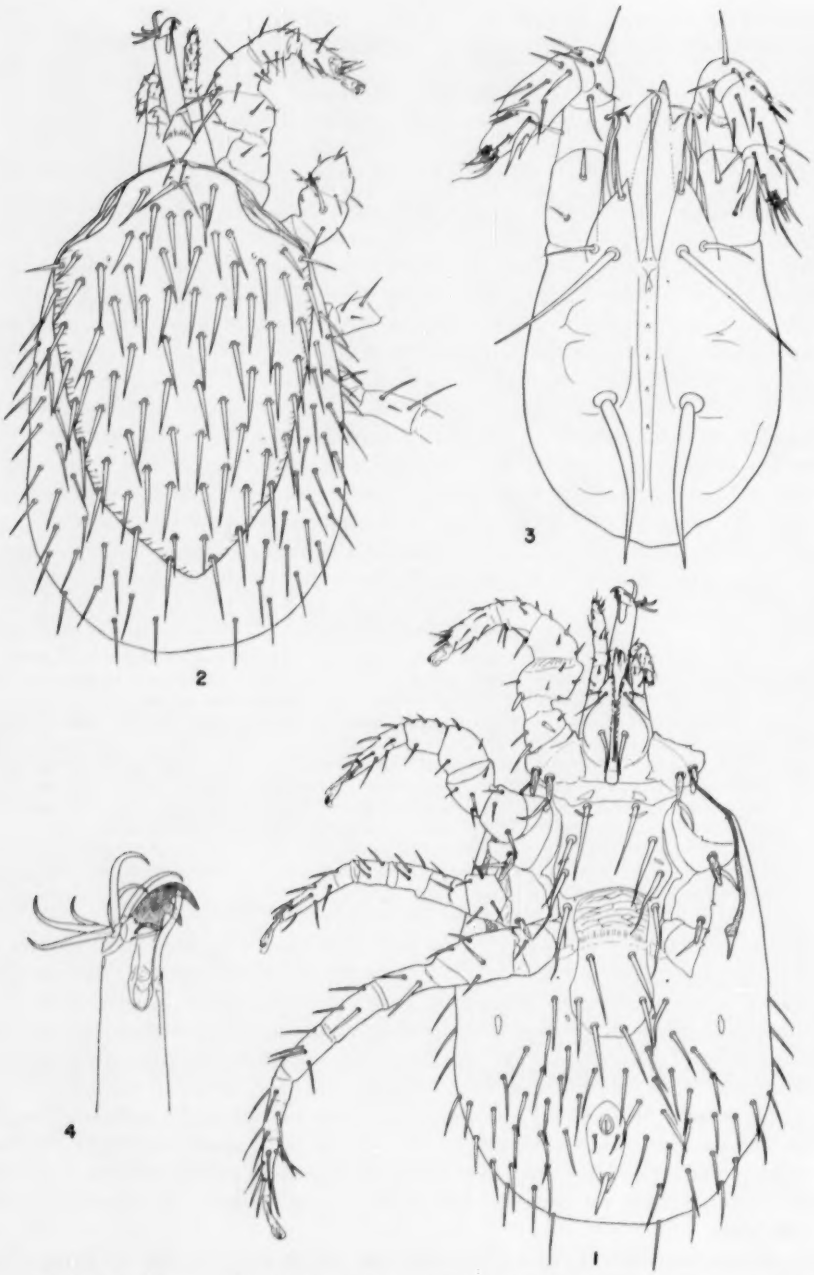
Genotype: *Steptolaelaps heteromydis* new species, described below.

Steptolaelaps is superficially similar to *Neolaelaps* Hirst, 1926. In *Neolaelaps* the peritreme is extremely broad and characteristic of the genus. The anterior ventral setae of coxae II and III are broad and spine-like. The chelicerae are markedly different from those of *Steptolaelaps*, having digits small, weak and untoothed in agreement with the description of Vitzthum (1926). Furthermore, *Neolaelaps* occurs on Malayan bats, *Steptolaelaps* on neotropical rodents.

Liponysella Hirst (1925) is also superficially similar to *Steptolaelaps*, but it is a dermanyssid mite. Examination of a cotype of *L. madagascariensis* (Hirst) revealed that it possesses untoothed chelicerae, relatively delicate setation, a strong antero-dorsal spur on coxa II, and numerous other features providing ready distinction.

Mesolaelaps Hirst (1926) is distinct from *Steptolaelaps* in that the coxal setae are all delicate, and the legs are long and slender.

All measurements given below are in microns.



Steptolaelaps heteromydis, female. FIG. 1. Venter; FIG. 2. Dorsum; FIG. 3. Ventral view of gnathosoma; FIG. 4. Chelicera.

Steptolaelaps heteromydis new species
Female, Figs. 1–4

Idiosoma broadly oval, 907 long by 475 wide at level of shoulders; 594 wide just posterior to coxae IV.

Dorsum—Dorsal plate widest at level of shoulders, measuring 778 long by 470 wide; acuminate posteriorly, leaving wide posterior and postero-lateral margins of hysterosoma uncovered; dorsal plate with prominent, antero-lateral, marginal sulcus on each side extending from mid-level of coxa I to posterior of coxa II. Plate with 40 pairs of setae, the anterior two pairs 48 long, others measuring up to 116 long; two elongate pores behind second pair of anterior setae. Uncovered portion of idiosoma bearing approximately 26 pairs of setae, more slender and somewhat shorter than average dorsal plate setae.

Venter—Tritosternum well developed with pair of laciniae narrowly elongate, pilose, divided almost to basal piece. Sternal plate sub-rectangular as illustrated, measuring 160 long at mid-line by 200 wide at level of second pair of setae; lateral borders fused with endopodal plates; usual two pairs of pores on sternal plate as figured, with a third pair of circular pores on unarmed cuticula antero-medial to metasternal setae. Genito-ventral plate reduced, bluntly angulate posteriorly, bearing three pairs of setae, the genital pair 145 long, approximately same size as sternal setae but longer than remaining genito-ventral setae. Anal plate narrowly elongate, boat-shaped, with pair of delicate adanal setae, 41 long, located at level of posterior anal margin; single postanal seta stout, 75 long. Metapodal plates small, irregularly elongate; two pairs of minute platelets near margins of genito-ventral plate, anterior ones circular, others elongate. Approximately 25 pairs of slender setae on unarmed, ventral opisthosoma, measuring up to 90 long. Peritreme narrow, extending anteriorly to level of posterior quarter of coxa I, and posteriorly to mid-level coxa IV.

Legs—As figured, stout and spinose; all legs with caruncles and paired claws. Coxa I bearing two stout, blunt, striated, ventral spines, a similar smaller spine on trochanter and also on femur ventrally; femur with two long, subapical dorsal spines; remaining setae not abnormal in appearance. Coxa II apparently without an antero-dorsal spur though small serrations present; anterior ventral seta not abnormally thickened but posterior ventral seta spine-like as on coxa I; femur with elongate dorsal setae similar to leg I but smaller; tarsus bearing three stout subapical spines in addition to normal setae. Coxa III setation similar to that of coxa II but smaller. Coxa IV bearing single ventral seta. Leg IV longer than other legs. Legs I and II stouter than III and IV.

Gnathosoma—Figs. 3–4 (Terminology as of Gorirossi and Wharton, 1953). Paired gnathosomal setae not spine-like, but elongate, extending beyond base of gnathosoma. Deutosternal groove bearing a single column of about five teeth with a minute distal pair between medial hypostomal setae; lateral and distal hypostomal setae much smaller than medial pair. Hypostomal processes sinuose with lateral lobes serrate apically and fused to two elongate medial processes with membranous recurved tips. Hypopharynx a narrowly elongated simple lobe with pointed apex, thickly covered with microchetae. Tectum an indistinct membranous lobe with small apical cleft and faint basal radiating striae. Chelicerae large with well developed movable digit bearing two strong teeth and ending distally in a recurved, claw-like tip; fixed digit a delicate, unarmed, thin lobe not reaching apex of other digit, with a short basal seta; base of movable digit surrounded by six prominent curved seta-like processes extending past tip of digit.

Male, Figs. 5–7

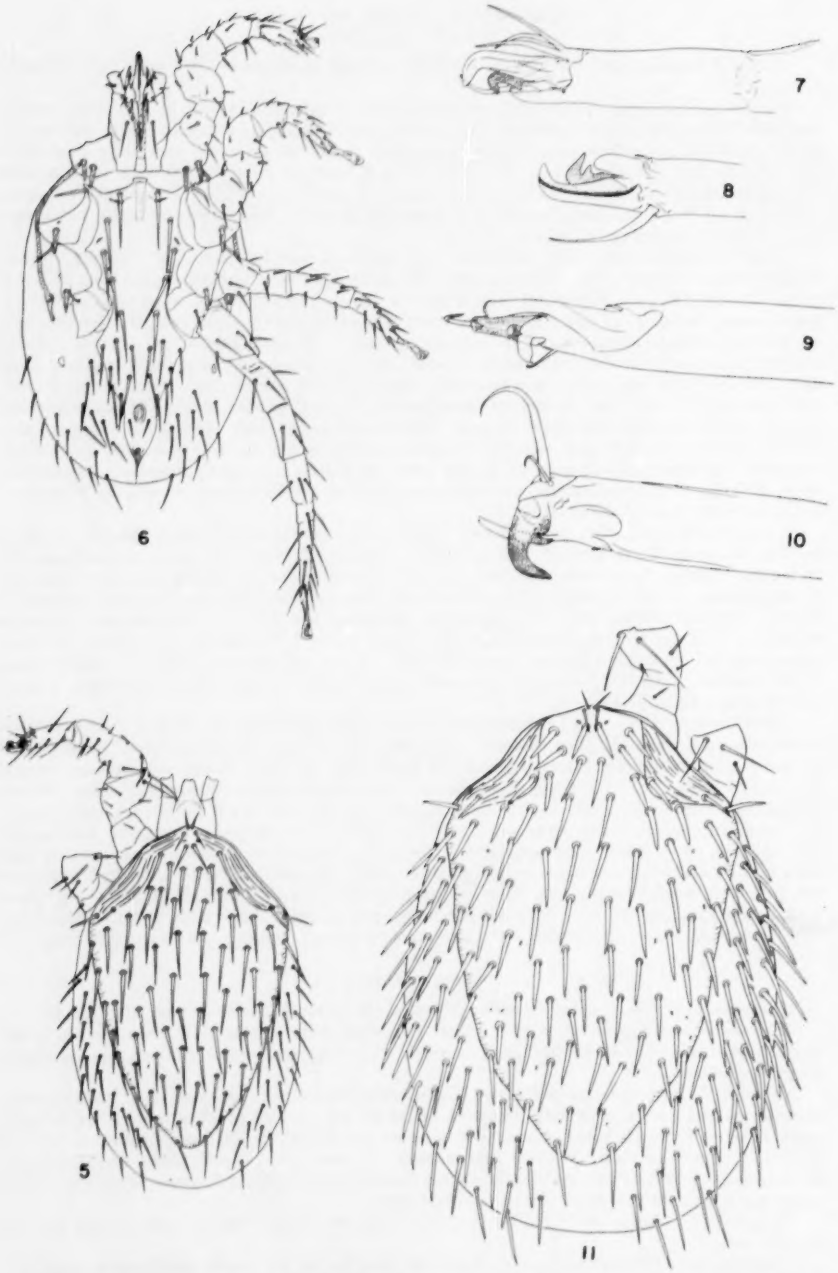
Idiosoma oval, 670 long by 378 wide at level of coxae II; 432 wide at level of coxae IV.

Dorsum—Dorsal plate of similar shape and setation as female; 648 long by 356 wide; longest setae 85 long, in central area of plate. Uncovered idiosoma bearing approximately 22 pairs of setae.

Venter—Tritosternum as in female. Holoventral plate entire, including anal plate; sternal, metasternal and genital setae approximately 105 long; ventral part of plate bearing six to seven pairs of setae, 73 to 85 long; adanal pair of setae small, delicate, 30 long, located at level of posterior margin of anus; postanal seta stouter, 62 long. Metapodal plates and peritremes as in female. Unarmed portion of opisthosoma bearing from seven to 10 pairs of strictly ventral setae, and approximately five pairs of marginal setae.

Legs—Essentially as described for female, except that tibia of leg II bears a stout, sinuous, ventral spine.

Gnathosoma—With exception of chelicerae, description of female gnathosoma applies to male. Chelicera with well developed movable digit bearing one strong tooth and terminating in a claw-like tip; fixed digit shorter, blunt, unarmed, concave medially, with a short, obscure seta arising near its base; a long, sheath-like structure, curved at tip arising behind movable



Steptolaelaps heteromydis, Figs. 5–7; Fig. 5. Dorsum of male; Fig. 6. Venter of male; Fig. 7. Chelicera of male. *Steptolaelaps liomydis*, Figs. 8–11; Fig. 8. Chelicera of male; Fig. 9. Ventral view of female chelicera; Fig. 10. Lateral view of female chelicera; Fig. 11. Dorsum of female.

digit and overshadowing it. Subtending base of sheath are six prominent curved seta-like processes approximately as long as the sheath.

Deutonymph

Idiosoma oval, 724 long by 420 wide.

Dorsum—Dorsal plate shape and setation similar to female, but proportionately smaller; plate 577 long by 334 wide. Unarmed portion of dorsum as in female.

Venter—Similar to female with following exceptions: Proportionately smaller. Sternal plate about 130 wide extending from anterior margin at level of anterior of coxae II to level midway between second and third pairs of setae, tapering thence to narrowly rounded posterior tip at level of posterior 1/3 of coxae IV; bearing four pairs setae and three pairs of pores; first three pairs setae 100 long, fourth pair 80 long; fifth pair of setae located adjacent to posterior borders of plate. Metapodal plates not visible. Unarmed opisthosoma with about 21 pairs of setae plus several marginal pairs.

Legs—Coxae as in female though stout spines more acutely pointed. Legs similar to those of female but spines not as stout.

Gnathosoma—As in female.

Holotype female collected July 27, 1950 from *Heteromys* sp. at Campamento Rafael Rangel, Aragua, Venezuela; allotype male taken from *Heteromys anomalus* (Thompson) at the same location and date (deposited in U. S. National Museum, No. 2215). The types and following paratypes from Venezuela were collected by J. M. Amberson, E. Schwarz and H. K. Schwarz of the U. S. Navy Venezuelan expedition. All paratype collections were from the same location as the holotype: 137 specimens from *Heteromys anomalus anomalus* (Thompson), 24 from *Heteromys* sp., 14 from *Sigmodon hispidus hirsutus* (Burmeister), four from *Rhipidomys nitela* Thomas, three from *Rattus rattus frugivorus* (Rafinesque), one from *Proechimys cayannensis trinitatis* Allen and Chapman and three from *Akodon urichi urichi* Allen and Chapman. In addition to the type series the author has seen six specimens of *S. heteromydis* from *Heteromys* sp. taken at Caracolicito, Magd., Colombia collected by P. Hershkovitz.

In examining the numerous specimens of *S. heteromydis* available, the characteristics described above seemed constant with a few minor exceptions. The number of setae on the ventral and dorsal sides of the idiosoma varied somewhat on the unarmed portions. Part of this is due to actual differences in numbers of setae but also partially to a degree of rotation of the cuticula caused by the pressure involved in mounting. The number of setae on the ventral portion of the male holoventral plate varies from six to seven pairs, and there may be six on one side and seven on the other.

S. heteromydis is distinct from *Steptolaelaps liomydis* (Grant). The female chelicera of the latter has a movable digit bearing only one tooth in addition to the terminal prong; it has only one long and one short seta-like process subtending the base of the movable digit; the male chelicera has only a single seta-like process arising near the base of the movable digit. The peritreme of both sexes of *S. liomydis* extends only to the posterior one half of coxae II; the gnathosomal setae of the female are short, stout and spine-like, the ventral and dorsal idiosomal setae are spine-like, stouter and less tapering than in *S. heteromydis*.

Steptolaelaps liomydis (Grant)

Laelaps liomydis Grant 1947, Microentomology **12** (1): 1–21, [Encarnacion de Diaz, State of Jalisco, Mex., *ex. Liomys irroratus;* type female lost, type male in coll. C. D. Grant].

The brief original description of this species was based on a single female and one male. Numerous specimens collected by the present author from *Liomys* spp. in Mexico agree in most respects with the original description which is here amplified and emended.

Female, Figs. 9–12

Idiosoma oval, 980 long by 568 wide at shoulders and 692 wide at level of coxae IV.

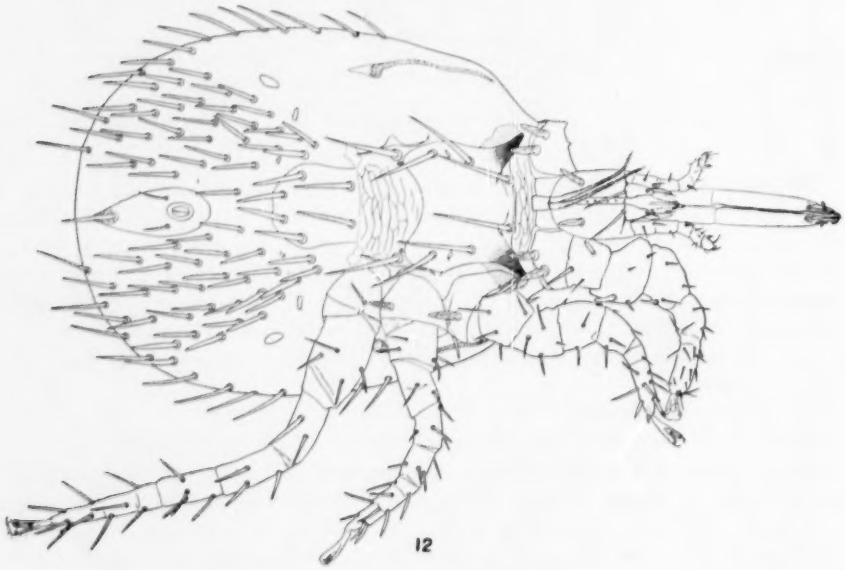
Dorsum—Dorsal plate widest at shoulders, measuring 880 long by 550 wide; acuminate

posteriorly leaving wide posterior and postero-lateral margins of hysterosoma uncovered; antero-lateral indentations of plate as in *S. heteromydis;* approximately 43 pairs setae, most of which stout and spine-like as figured; anterior two pairs 46 long, others up to 106 long. Unarmed dorsum bearing approximately 46 pairs setae, several marginal.

Venter—Tritosternum as in *S. heteromydis.* Sternal plate sub-rectangular, concave posteriorly as figured, measuring 208 long at midline by 225 wide at level of second pair of setae; antero-lateral corners of sternal plate and supporting subcuticular apodemes heavily sclerotized. Genito-ventral plate as in *S. heteromydis* but setae shorter and stouter. Anal plate narrow, elongate, boat-shaped; adanal setae much smaller than postanal seta. Metapodal plates elongate oval; two pairs of smaller platelets located as figured. Approximately 35 pairs setae on unarmed opisthosoma plus a variable number of marginal pairs, stouter than in *S. heteromydis.* Peritreme narrow, extending anteriorly to posterior half of coxa II, posteriorly to rear one fourth of coxa IV.

Legs—As described for *S. heteromydis.*

Gnathosoma—As described for *S. heteromydis* with following exceptions: Paired gnathosomal setae short, broad spines resembling those of coxa I; chelicerae (Figs. 9–10) large with well-developed movable digit claw-like apically and with one strong tooth; fixed digit an unarmed thin lobe with a short basal seta; a membranous acutely tipped leaf-like structure apparently rising between digits; one long and one short seta-like process arising lateral to base of movable digit.



Steptolaelaps liomydis. FIG. 12. Venter of female.

Male

Idiosoma oval, 605 long by 405 wide at level of coxae IV.

Dorsum—Dorsal plate shape as in female; 590 long by 368 wide; bearing 41 pairs setae. Uncovered dorsum bearing about 27 pairs of setae.

Venter—Tritosternum as in female. Holoventral plate entire including anal plate; sternal and metasternal setae about 100 long, genital setae 94 long; ventral part of plate with six to seven pairs of setae from 67 to 77 long; adanal pair of setae small, delicate; postanal seta stout, 60 long. Paired metapodal plates and peritremes as in female. Unarmed opisthosoma bearing about 17 pairs of setae.

Legs—As described and figured for female except that femur and tibia of leg II each bear a short blunt ventral spine.

Gnathosoma—With exception of chelicerae, description of female gnathosoma applies to

male. Chelicera (Fig. 8) with movable digit as in female but smaller; fixed digit an unarmed lobe half the length of movable digit and bearing a small seta at the base; a long, broad, sheath-like structure curved at tip arising behind movable digit and overshadowing it; subtending base of sheath is a single prominent, curved seta-like process almost as long as sheath.

The above description is based on a female and male from *Liomys irroratus acutus* Hall and Villa, taken near Patzcuaro, Michoacan, Mexico, Nov. 10, 1953 by the author. The male was compared with the type and found to be identical. Other specimens collected by Furman and Price in Mexico from August through November, 1953 and compared with the above description are as follows: Many from *Liomys irroratus irroratus* (Gray) taken on Cerro San Felipe and Monte Alban near Oaxaca, Oax.; from *Liomys pictus isthmius* Merriam near Tuxtla, Chiapas and at the crossing of the Rio San Gregorio by the Pan-American highway, Chiapas, approximately 20 miles from Guatemala; from *Liomys irroratus acutus* Hall and Villa near Patzcuaro, Michoacan. Several specimens were also seen from *Liomys pictus veraecrucis* Merriam taken by Barrera at San Andres Tuxtla, Vera Cruz, and a single specimen from *Liomys pictus parviceps* Goldman taken by Wharton at Coalcoman, Michoacan. Through the courtesy of R. W. Strandtmann three specimens of *S. liomydis* were seen, taken from *Liomys irroratus* in Hidalgo Co., Texas. These represent the only specimens reported from the United States.

The above descriptions compare favorably with the original descriptions except as follows: In the female the anal plate is rather acutely rounded anteriorly; leg I bears a stout ventral spine on both trochanter and femur; the chelicerae have only the movable digit toothed; the "palmate seta" of Grant appears to be a leaf-like membranous lobe arising between the two digits. In the male the holoventral plate includes the anal plate; the elongate fixed digit of the chelicera illustrated by Grant is here interpreted as the sheath of the movable digit; the fixed digit is actually a short unarmed lobe as figured here, subtended by one short basal seta; there is only one long seta, which rises basal to the sheath.

SUMMARY

A new genus, *Steptolaelaps* (ACARINA: LAELAPTIDAE), is established for two species of mites parasitic on neotropical rodents. *Steptolaelaps heteromydis* is described as a new species and type of the genus, occurring primarily upon *Heteromys* spp., and is represented by collections from Venezuela and Colombia. *Steptolaelaps liomydis* (Grant) is redescribed. It is primarily parasitic upon *Liomys* spp., being very common on these rodents in Mexico.

LITERATURE CITED

GORIROSSI, F. AND G. W. WHARTON '1953 The anatomy of the feeding apparatus of *Megisthanus floridanus* Banks, 1904, a large mesostigmatid mite. Am. Midl. Naturalist **50** (*2*): 433–447.

HIRST, S. 1925 Descriptions of new Acari, mainly parasitic on rodents. Proc. Zool. Soc. London, Pt. 1, Apr., pp. 49–69.

———1926 Descriptions of new mites, including four new species of "red spider." Proc. Zool. Soc. London, Part III, Sept., pp. 825–841.

VITZTHUM, GRAF. H. 1926 Malayische Acari. Treubia **8** (1-2): 1–198.

———1942 Acarina. Bronn's Klass. u. Ordnung. Tierreichs. v. **5** (Abt. 4, Buch 5, Lief. 5): 768–769.

THE ESTABLISHMENT OF A STRAIN OF *AUSTRALORBIS GLABRATUS* WHICH COMBINES ALBINISM AND HIGH SUSCEPTIBILITY TO INFECTION WITH *SCHISTOSOMA MANSONI*

WALTER L. NEWTON[1]
U. S. Department of Health, Education, and Welfare
Public Health Service
National Institutes of Health
National Microbiological Institute, Laboratory of Tropical Diseases
Bethesda, Maryland

Among the strains of *Australorbis glabratus* maintained in this Laboratory are an albino Brazilian strain which is not susceptible to infection with a Puerto Rican strain of *Schistosoma mansoni* and a pigmented Puerto Rican one which is highly susceptible. When these snails at a size of 8 to 10 mm. were exposed to 10 miracidia, 0% and 95% respectively, of the albino and the pigmented strains became infected with cercariae (Newton, 1953). Studies of crosses between these strains have demonstrated that this susceptibility is inherited as a multifactor character (Newton, 1953). It was also shown that albinism in *A. glabratus* is inherited as a simple Mendelian recessive (Newton, 1954). It was of interest to attempt to combine these characters and develop an albino strain which was also highly susceptible to the parasite. Such snails would be useful in further genetic studies, and because of the transparency of the albino, certain phases of the development of the parasite might be followed in the live snail.

MATERIALS AND METHODS

The procedure followed was relatively simple and essentially required only careful observation and attention. Starting with the pigmented offspring of an albino nonsusceptible snail which had been inseminated by a pigmented susceptible one (F_1), a few snails of each generation were isolated individually in beakers from the time they were 1 to 1.5 mm. in size. They were maintained in this manner until one of them produced, through self-fertilization, sufficient offspring to ensure a subsequent generation, at which time its siblings were discarded. This was continued for 10 consecutive generations of selfing, a procedure which is presumed to result in a high degree of homozygosity.

The parent of the 4th generation was selected for susceptibility, and those of the 5th and 6th generations for both albinism and susceptibility. Selection for susceptibility was carried out in the following manner: Offspring obtained from the several isolated individuals of a generation were kept until the latter were exposed to infection and checked for susceptibility. An isolated offspring of one of the positive snails was then chosen for continuation of the line. The other parents and their offspring were discarded. From the 6th generation on, no selection was made; all subsequent snails were albinos and no check was made of their susceptibility. One of the snails chosen randomly from the 10th generation produced about 40 offspring from which a colony was established, approximately 3 years after the initial cross. A resumé of the history of this cross is presented in Table 1.

Received for publication, April 5, 1955.

[1] With the technical assistance of Mr. Paul C. Shade.

TABLE 1.—*History of the development of an albino strain of* Australorbis glabratus *which is susceptible to* Schistosoma mansoni

Generation	Description of parent of subsequent generation	Selection, if any
Cross	*a*lbino *R*esistant crossed with *P*igmented *S*usceptible aR × PS	None
F_1	PR	None
F_2	PR	None
F_3	PS	Selected for S
F_4	aS	Selected for aS
F_5	aS	Selected for aS
F_6	a?	None
F_7–F_{11}	a?	None
aS colony established		

RESULTS AND DISCUSSION

Several lines were initiated and maintained to ensure the establishment of at least one, because reproduction and growth were quite variable. For reasons which were not apparent, and which might be associated with the high degree of inbreeding, many of the isolated snails failed to produce eggs, or the latter failed to develop and hatch properly. Some lines progressed rather vigorously for several generations, and then could not be continued. Even when the colony was initiated and crossbreeding again became possible, it was several months before the former was well-established.

As soon as samples could be spared, the snails of this colony were tested for susceptibility to infection with the strain of *S. mansoni* to which the original parent albino strain was refractory. In three tests, a total of 113 snails of a size of 8 to 10 mm. was exposed to 10 miracidia each, and 107 of these survived the developmental period of the parasite. Of the survivors, 91 (85%) were positive. Twelve of the 16 negatives were subsequently reexposed to the parasite, four

FIG. 1. Sporocyst of *Schistosoma mansoni* in mantle of live albino *Australorbis glabratus* ×90.

having died in the interim. Of the 12 exposed a second time, 11 became positive for cercariae. Development of the parasite in this strain did not appear to differ from that in the susceptible pigmented parent strain. The majority of the snails shed cercariae 4.5 to 5 weeks after exposure and maintenance at temperatures of 72° to 75° F.

As an illustration of the transparency of this snail, figure 1 is a photomicrograph of a sporocyst in the mantle of a live snail, as seen through the shell by means of transmitted light from a microscope lamp. The parasite was located just anterior to the heart, the structure and activity of which, incidentally, are readily visible and appear to be suitable for other studies such as the effects of molluscacides.

It is apparent that we have developed a strain, or population, which is different from either of the original parent strains in that it is composed of susceptible albino snails. Presumably, this strain is genetically rather homogeneous, although it is to be expected that with random breeding and the occurrence of mutations there will be a gradual loss in this high degree of homozygosity. However, because of the probability that several genetic factors are involved, it seems likely that this strain will retain its rather uniform susceptibility for a considerable period.

SUMMARY

An albino strain of *Australorbis glabratus* which is highly susceptible to a Puerto Rican strain of *Schistosoma mansoni* has been developed from pigmented susceptible and albino resistant strains. The new strain was established as a colony following 10 consecutive generations of selfing after the initial cross between the parent strains. Small daughter sporocysts can be seen moving about in the live infected snail because of the relative transparency of the latter.

REFERENCES CITED

NEWTON, W. L. 1953 The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus*. Exptl. Parasitol. **2**: 242–257.

———1954 Albinism in *Australorbis glabratus*. Proc. Helm. Soc. Wash. **21**: 72–74.

THE DISTRIBUTION OF LARVAL STAGES OF *ASPICULURIS TETRAPTERA* IN THE INTESTINE OF MICE

K. F. CHAN
School of Public Health, College of Physicians and Surgeons, Columbia University

For several years investigators in the Department of Parasitology, School of Public Health, Columbia University have been evaluating the anthelmintic activity of numerous compounds against the mouse oxyurids *Syphacia obvelata* and *Aspiculuris tetraptera,* (Wells, 1952; Chan, 1952; Hsieh, 1952; Brown, Chan and Ferrell, 1954). The methods employed in these chemotherapeutic studies were subject to changes and revisions with the addition of knowledge of the life history and host-parasite relationship of the worms. Recently the efficacy of a group of piperazine compounds against *S. obvelata* and the specific action of piperazine hydrochloride on the immature and mature stages of this parasite have been reported by Brown, Chan and Hussey (1954). These studies confirmed the clinical impression of Mouriquand and associates (1951) that gravid *Enterobius* appear to be more susceptible to piperazine hydrate than the immature forms. These studies have also been extended to several developmental stages of *A. tetraptera,* and the results indicate that while piperazine hydrochloride is markedly active against *Aspiculuris,* it did not show the specificity of action against mature worms that it did against *Syphacia* (data unpublished).

During tests of chemotherapeutic agents against immature *Aspiculuris,* it was soon recognized that these larval forms are distributed throughout the mouse's intestine as was noted by Hall (1916) and Philpot (1924). It appeared, however, that the distribution of the worms formed a distinct pattern related to their age. The object of the present study was to search for these early larval stages and to establish the distribution pattern of the worms in the intestine of their host.

MATERIALS AND METHODS

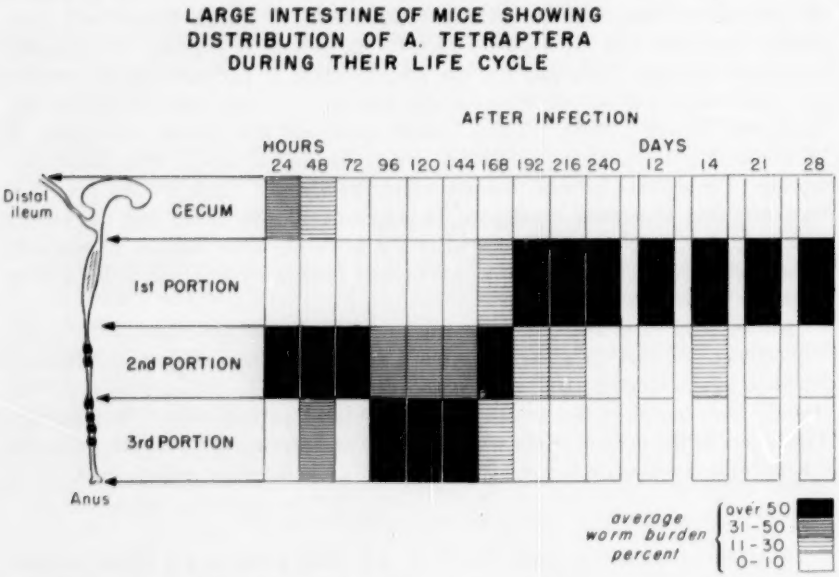
In order to obtain significant results in this study employing a limited number of animals, it was essential to achieve uniform infections in the animals. Hence, a single source of stored infective eggs of *A. tetraptera* was used for the entire study, as Chan (1953) has demonstrated the uniform infectivity of refrigerated *Aspiculuris* eggs. Nematode-free male mice, 5 to 6 weeks old, were employed, as Mathies (1954) noted the marked differences in the experimental infection rates in male and female mice of the same age. The mice were given an infecting dose of 200 to 700 eggs each. This range of infection was intended to provide both light and heavy infections for study. At 24-hour intervals for the first 10 days and at longer intervals thereafter, 3 to 5 mice were autopsied and the intestine was cut into numerous sections as follows: the distal ileum, the cecum, the proximal 1 inch of the colon below the cecum, and the two remaining portions of the colon respectively, (Graph I). These sections were then opened in shallow dishes containing zinc sulphate

Received for publication, March 8, 1955.
The author wishes to thank Dr. H. W. Brown for his generous help in designing these experiments. He also wishes to acknowledge the valuable technical assistance of Miss Sydell Kopilof in this study.
This investigation was supported in part by a research grant E-461 (R) of the National Institutes of Health, Public Health Service.

solution and were carefully scraped in order to dislodge the larvae into the solution where they could be counted under a dissecting microscope. The pieces of slit-open intestine were further examined for larvae by the slide compression technique, since the worms may adhere closely to the mucosal wall. Measurements of the length of 10 developing larvae were taken from each section of the intestine whenever possible.

RESULTS AND DISCUSSION

As early as 24 hours after the feeding of infective *Aspiculuris* eggs to mice, larvae were found in the whole length of the large intestine, (Table I and Graph I).



However, 72 to 144 hours after infection, larvae of *Aspiculuris* were primarily located in the lower colon of the host and were very rare in the cecum and the 1st portion below the cecum. Larvae in the lower colon and those in proximity to the anus, apply themselves closely to the mucosa presumably to retain their position in the intestine, as the fecal material at this level is relatively dry. Beginning the 8th day (168 hours) after infection, larval forms reappeared in the proximal portion of the large intestine in increasing numbers, and at the same time their numbers diminished gradually in the lower colon. By the 11th day (240 hours) after infection and thereafter as late as the 29th day of observation, the majority of the worms were found in the 1st portion of the colon and frequently were totally absent in the 3rd portion. The degree of infection did not appear to influence the pattern of distribution of these immature worms in the host's intestine.

The experimental results indicate that larvae of *A. tetraptera* first migrate downwards in the mouse's intestine as reported by various observers and subsequently migrate back up the colon to reach their natural habitat in the proximal section of the large intestine. Wells (1952) and Hsieh (1952) observed that these larvae

migrated from the cecum to the colon of mice 24 hours after infection, but Philpot was of the opinion that migration of *Aspiculuris* larvae did not occur until after 90 hours. It is evident from the present study that immature *Aspiculuris* still remained in the host's cecum as late as 96 hours after infection. Both Philpot and

TABLE I.—*The distribution of* A. tetraptera *in the intestine of mice at varying periods after infection*

Time after infection hours, days		Mouse no.	WORM BURDEN						Average length larvae mm.
			Distal ileum	Large intestine					
				Cecum	Portions below Cecum				
					1st	2nd	3rd	TOTAL	
24	(1)	1	0	7	0	60	0	67	0.11
		2	1	70	3	21	1	96	
		3	0	12	1	86	2	101	
48	(2)	1	0	32	3	40	37	101	0.19
		2	0	28	16	76	8	128	
		3	0	0	0	72	59	131	
72	(3)	1	0	30	13	78	13	134	0.21
		2	0	0	0	152	3	155	
		3	0	0	0	146	16	162	
96	(4)	1	0	0	1	16	92	108	0.22
		2	0	0	0	54	66	120	
		3	0	10	0	98	52	160	
		4	0	0	0	90	78	168	
120	(5)	1	0	0	0	18	86	104	0.37
		2	0	0	0	3	107	110	
		3	0	0	0	45	84	129	
		4	0	0	0	27	111	138	
		5	0	0	0	150	54	204	
144	(6)	1	0	0	1	57	26	84	0.44
		2	0	0	5	34	70	109	
		3	0	0	4	60	70	134	
		4	0	0	0	94	88	182	
168	(7)	1	0	7	2	82	1	92	0.61
		2	0	1	5	96	52	154	
		3	0	1	2	105	50	158	
		4	0	0	40	59	59	198	
192	(8)	1	0	0	40	34	0	74	0.75
		2	0	3	117	45	0	165	
		3	0	0	143	22	27	190	
		4	0	5	165	125	28	323	
216	(9)	1	0	0	67	15	3	85	—
		2	0	3	124	20	0	147	
		3	0	0	179	17	10	206	
		4	0	14	183	19	0	216	
240	(10)	1	0	0	95	7	0	102	0.87
		2	0	5	167	28	0	200	
		3	1	19	242	1	0	263	
288	(12)	1	0	0	84	5	0	89	1.1
		2	0	2	115	2	0	119	
		3	0	1	452	47	2	502	
		4	0	5	437	61	0	503	
336	(14)	1	0	0	67	17	8	92	—
		2	0	2	127	42	0	171	
		3	0	0	286	3	0	289	
504	(21)	1	0	5	106	1	0	112	—
		2	0	0	200	3	0	203	
		3	0	8	202	1	0	211	
672	(28)	1	0	12	126	0	0	138	—
		2	0	5	430	6	0	441	
		3	0	30	439	11	0	480	

Hsieh recognized that larvae were rare in the cecum at 90 hours; the former investigator stated that at 163 hours after infection larvae of *A. tetraptera* occurred mainly in the large intestine of mice in the area one-half to one inch below the cecum. This segment of the colon designated in the present paper as the 1st portion for the purpose of description is somewhat thickened and dilated due to the presence of prominent rugae, and it apparently is the chief habitat of *Aspiculuris*. Wells, though unable to observe the actual embedding of the parasite in the intestinal mucosa, through serial histological sections, believed that *Aspiculuris* probably gain some anchorage within these prominent intestinal folds.

The phenomenon of a downward migration followed by an upward migration of larval *Aspiculuris* in the intestine of mice may have practical significance in chemotherapy. The immature forms of the parasite may therefore possibly be less subject to the action of chemotherapeutic agents because of their habitat low in the host's colon. The upward migration of *Aspiculurus* provides support for retrofection as a mode of infection in the human species, *Enterobius vermicularis* (Schüffner & Swellengrebel, 1949). The varied locations of the *Aspiculuris* worms in the early stages, too, pose an additional difficulty in their recognition and enumeration when these stages are employed for chemotherapeutic studies.

SUMMARY

The distribution of larval stages of *Aspiculuris tetraptera* in the intestine of infected mice has been described, and a preliminary downward migration followed by an upward migration of the immature stages is reported. This migration is perhaps of importance in experimental chemotherapy of *Aspiculuris*.

REFERENCES CITED

BROWN, H. W., CHAN, K. F., AND FERRELL, B. D. 1954 A study of the activity of chemotherapeutic agents on infections of *Syphacia obvelata* and *Aspiculuris tetraptera*. Exper. Parasitology **3**: 45–51.

BROWN, H. W., CHAN, K. F., AND HUSSEY, K. L. 1954 The efficacy of piperazine compounds against *Syphacia obvelata* a pinworm of mice. Am. J. Trop. Med. & Hyg. **3**: 504–510.

CHAN, K. F. 1952 Chemotherapeutic studies on *Syphacia obvelata* infection in mice. Am. J. Hyg. **56**: 22–30.

———1953 The effect of storage at low temperatures on the infectivity of *Aspiculuris tetraptera* eggs. J. Parasit. **39** (No. 4, Sect. 2): 42.

HALL, M. C. 1916 Nematode parasites of mammals of the orders Rodentia, Lagomorpha and Hyracoidea. U. S. Nat. Mus. **50**: 1–258.

HSIEH, K. Y. N. 1952 The life cycle of *Aspiculuris tetraptera* and its relationship to chemotherapeutic agents. Doctoral thesis, Columbia University.

MATHIES, A. W. 1954 The influence of sex on mouse pinworm infection. J. Parasit. **40**: 702.

MOURIQUAND, G., ROMAN, E., ET COISNARD, J. 1951 Essai de traitement de l'oxyurose par la piperazine. J. Med. Lyon **32**: 189–195.

PHILPOT, F. 1924 Notes on the eggs and early development of some species of Oxyuridae. J. Helminthol. **2**: 239–252.

SCHÜFFNER, W. AND SWELLENGREBEL, N. H. 1949 Retrofection in oxyuriasis. A newly discovered mode of infection with *Enterobius vermicularis*. J. Parasit. **35**: 138–146.

WELLS, H. S. 1952 Effect of antibiotics on infections with the mouse pinworm *Aspiculuris tetraptera*. Doctoral thesis, Columbia University.

NOTES ON THE GENUS *MARITREMA* NICOLL, 1907 (TREMATODA: MICROPHALLIDAE) WITH THE DESCRIPTION OF TWO NEW SPECIES

WILLIAM H. COIL
Department of Zoology and Entomology
The Ohio State University

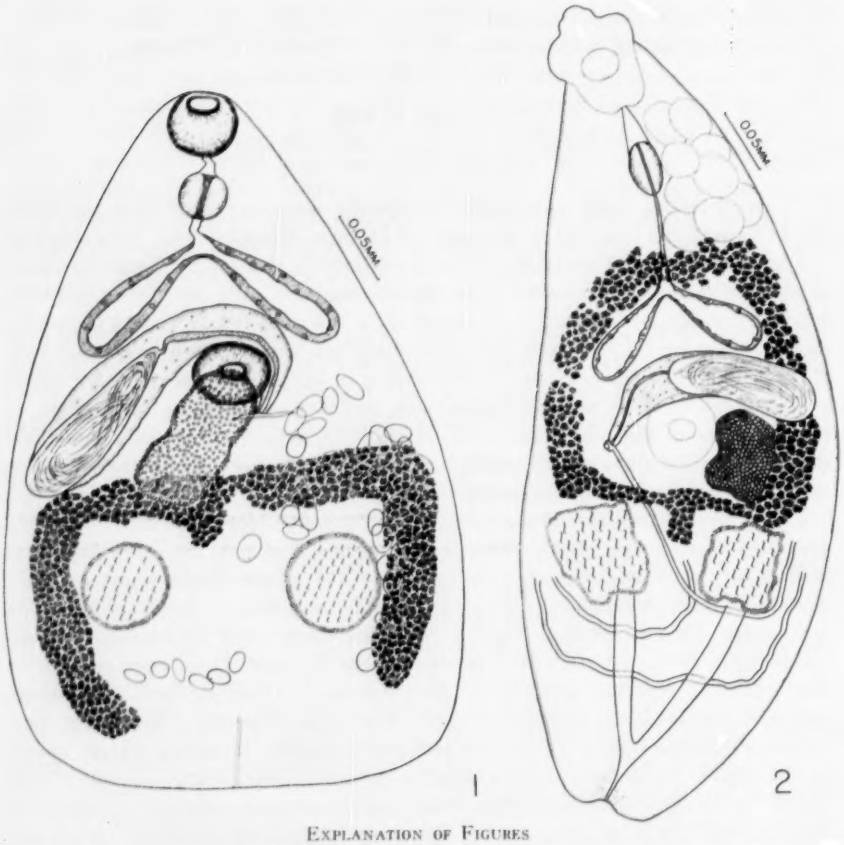
Two species of small microphallid trematodes were recovered from the intestines of two birds collected in the state of Oaxaca, Mexico. Two individuals of one species infected a sandpiper (*Tringa solitaria**), and only a single specimen of another species was collected from the intestine of a little blue heron (*Florida caerulea caerulea*). The family to which these distomes belong occurs in several classes of vertebrates, but the genus to which they can be assigned, *Maritrema* Nicoll, 1907, is found mainly in birds. The presence of one of these species in a heron is considered to be of no great importance, however, since it has been noted by other authors (Cable and Kuns, 1951, Baer, 1943, and Rausch, 1947) that these distomes are not greatly host-specific and that they have precocious metacercariae which can rapidly develop to maturity.

Representatives of species now assigned to the genus *Maritrema* occur in widely separated parts of the world. This is not unusual, however, since the groups of birds which serve as hosts are cosmopolitan. The adults of the genus are very similar in their morphology. This close similarity, alone, renders separation of the species difficult, but this difficulty is further complicated by the lack of host-specificity and by variation in structures generally used as taxonomic criteria. Etges (1953) reported variation in the disposition of the vitellaria and uterus, and also disparity in the sizes of suckers in *Maritrema obstipum*. Considering these variations and the nature of the terminal male genitalia, he added several species to the genus *Maritrema*. His summation presents these changes briefly: "The genus *Pseudospelotrema* Yamaguti, 1939 is suppressed as a synonym of *Maritrema* Nicoll, 1909 (*sic*), and the species *Pseudospelotrema japonicum, P. uriae,* and *P. cincli* are transferred to *Maritrema* as *Maritrema japonicum, M. uriae,* and *M. cincli.* The genus *Maritreminoides* Rankin, 1939 is restored to contain *Pseudospelotrema nettae* and *P. ammospizae* as *Maritreminoides nettae* (type) and *M. ammospizae. Pseudospelotrema obstipum* and *P. medium* are returned to the genus *Maritrema.*" When one considers the nature of the variations in *M. obstipum* which are pointed out by Etges, it is possible to assign both species studied here to the genus *Maritrema.*

The specimens were fixed in corrosive acetic (saturated $HgCl_2$ and 10 percent glacial acetic acid), stained with Harris' haematoxylin, and cleared in terpineol. All measurements are in millimeters. This study was carried out while the author was a Muellhaupt Scholar in the Department of Zoology and Entomology, The Ohio State University. The author is indebted to Allen McIntosh for the prompt loan of the following specimens from the U. S. National Museum collection: *Maritrema obstipum, M. medium,* and *Pseudospelotrema ammospizae.*

Received for publication, April 6, 1955.

* Identified by Jeff Swinebroad, Department of Zoology and Entomology, The Ohio State University.



EXPLANATION OF FIGURES

FIG. 1. Ventral view of *Maritrema patulus* drawn with the aid of a microprojector.
FIG. 2. Dorsal view of *Maritrema glandulosa* drawn with the aid of a microprojector.

Maritrema glandulosa sp. nov.
Fig. 2

Diagnosis: with characters of the genus. Small, completely spinose distomes, body 0.58 long, 0.23 wide at acetabulum. Oral sucker 0.064 by 0.075; acetabulum 0.051, located centrally with cirrus-sac curved around anterior margin. Prepharynx 0.024 long with gradually enlarging lumen. Pharynx 0.043 by 0.024. Esophagus 0.045 long. Ceca 0.098 and 0.093 long, walls heavy, irregular in thickness, containing large nuclei. Cirrus-sac arcuate, long, extending almost to right side of worm. Wall of cirrus-sac thin, containing prostate gland and large, seminal vesicle filled with sperm. Ductus ejaculatorius thin-walled, slightly coiled, terminating in a "papilla." Ovary dextral, 0.048 by 0.060. Testes of irregular shape, 0.057 by 0.058, and 0.053 by 0.061, more or less symmetrically placed. Testes 0.13 and 0.15 from posterior end. Vitelline follicles arranged in ring anterior to testes and enclosing cirrus-sac, acetabulum, ovary, and ceca. Vitelline follicles 0.01 to 0.03 wide. Anterior vitellaria 0.17 from anterior end; posterior vitellaria 0.18 from posterior end. Excretory bladder V-shaped, arms measuring 0.016 and 0.022 in maximum diameter. Body filled with large, unicellular glands opening to exterior over most of body surface through small, slightly convoluted ducts. Eggs measuring 0.013 by 0.022.

Host: Little blue heron (*Florida caerulea caerulea*).

Locality: In the region of Tututepec, Oaxaca, Mexico.

Type specimen: Holotype in Helminthological Collection of the U. S. National Museum, No. 37471.

Although the specimen is young (a single egg had developed), and it is likely that it would have become larger, there is no reason to believe that the characters described here would change significantly with the expected increase in size. Actually, Hadley and Castle (1940) depicted the metacercaria of *Maritrema arenaria* as well-developed and the sexual adult as little changed from the metacercarial condition.

Maritrema obstipum, M. medium and *M. caridinae* all are similar to *M. glandulosa,* but the latter differs from them by possessing the following combination of characters: 1) a slightly lobed ovary; 2) large, unicellular glands in parenchyma; 3) oral sucker larger than the ventral sucker; 4) vitellaria situated in a median ring composed of small follicles. It must be remembered, however, that the disposition of the vitellaria may be aberrant in this case, and it may be that in other specimens of this species the vitellaria may be found in the posterior part of the body.

Maritrema patulus sp. nov.
Fig. 1

Diagnosis: with the characters of the genus. Small, subdeltoid distomes with completely spinose cuticula. Body 0.43 long and 0.29 wide. Oral sucker terminal, 0.048 by 0.038; acetabulum 0.057 in diameter, located 0.16 from anterior end. Prepharynx 0.009 long. Ceca short, capitate, extending to level of anterior margin of acetabulum. Walls of ceca irregular in thickness. Cirrus-sac extremely prominent, 0.2 long, arcuate distally, extending to right side of worm and encircling left side of acetabulum. Cirrus-sac thin walled, enclosing prostate glands and large seminal vesicle filled with spermatozoa. Ductus ejaculatorius only slightly coiled, thin-walled and terminating in "papilla" bearing very few minute, rectangular spines. Genital pore posterosinistral, near acetabulum. Vagina thin-walled, leading posteriorly from genital pore. Ovary posterodextral to acetabulum, strongly lobed dorsoventrally, 0.09 by 0.05. Testes subcircular, 0.067 by 0.073, and 0.060 by 0.054, more or less symmetrically placed. Vitelline follicles arranged in incomplete ring posterior to and enclosing testes. Excretory bladder thin-walled, Y-shaped. Eggs variable in size, 0.012 to 0.021 by 0.010 to 0.014. Eosinophilic glands present in parenchyma, moderate in size and number.

Host: Sandpiper (*Tringa solitaria*).

Locality: In the region of Salina Cruz, Oaxaca, Mexico.

Type specimens: Holotype in the Helminthological Collection of the U. S. National Museum, No. 37472.

Maritrema patulus possesses the following combination of characters by which it can be differentiated from the other members of the genus: 1) completely spinose cuticle; 2) strongly lobed ovary; 3) large cirrus sac; 4) genital pore posterosinistral to the acetabulum. *M. erolae* is the most closely related form, but differs by the possession of a heavy-walled vagina. The body shape is probably characteristic, but figures, for comparison, were not included with the descriptions of several of the species.

An examination of Table I yields several things of importance to one classifying distomes in this genus. With the exception of *M. pulcherrima,* which possesses an unusually large oral sucker, the suckers of most of the species are roughly proportional to the body size. There is approximately a three-fold difference in the ranges of the maximum body lengths and the maximum sizes of the oral suckers. The maximum egg lengths range from 0.016 to 0.029, the mean (of the maxima) is 0.021 and the median is only slightly less. From these size differences and their ranges, it is apparent that size alone, in most cases, is almost useless as a taxonomic criterion. More valid criteria, probably, are the other structures listed in the table (vagina, spination, and the relative arrangement of the ovary, genital pore, and acetabulum). Although Etges (1953) noted variation in the disposition of the

TABLE I. *The arrangement of the ovary, genital pore, and acetabulum was taken from the descriptions, not drawings, in the following species:* Maritrema gratiosum, M. lepidum, M. oöcysta, *and* M. rhodanicum. *Dimensions are in millimeters.*

MARITREMA	LENGTH	WIDTH	ORAL SUCKER	VENTRAL SUCKER	VITELLARIA	EGG	VAGINA	SPINES	GENITAL PORE
GRATIOSUM NICOLL, 1907	0.45-1.10	0.24-0.44	0.043-0.062	0.043-0.062	POSTERIOR RING	0.020-0.022		MANY	
LEPIDUM NICOLL, 1907	0.45-1.10	0.24-0.44	0.068	0.059	POSTERIOR RING	0.018-0.019	Z-SHAPED	MANY	
OÖCYSTA FROM NICOLL, 1907	0.28-0.40	0.12-0.18	0.025-0.031	0.030-0.034	POSTERIOR RING	0.016-0.018		ALMOST COVERED	
LINGUILLA JAGERSKIÖLD, 1909	0.44-0.53	0.19-0.22	0.036-0.039	0.048-0.054	POSTERIOR RING	0.015-0.016	HEAVY-WALLED	TO TESTES	
SUBDOLUM JAGERSKIÖLD, 1909	0.35-0.49	0.20-0.25	0.031-0.035	0.042-0.054	POSTERIOR RING	0.019-0.021	HEAVY-WALLED	PAST MIDWAY	
NICOLLI TRAVASSOS, 1921	0.35-0.50	0.21-0.26	0.03-0.04	0.04-0.05	POSTERIOR RING	0.019-0.021			
PULCHERRIMA TRAVASSOS, 1929	0.40-0.52	0.32-0.38	0.08-0.10	0.04-0.048	INCOMPLETE RING	0.018-0.020	HEAVY-WALLED	TOTAL	
SACHALINIUM SCHUMAKOWSTSCH	0.98-1.2	0.43-0.53	0.061-0.087	0.061-.087	POSTERIOR RING	0.020-0.02		HALF	
OBSTIPUM FROM ETGES 1953	0.48	0.28	0.06	0.06	RING	0.019-0.024	THIN-WALLED	TOTAL	
MEDIUM VAN CLEAVE & MUELLER, 1932	0.51-0.60	0.33-0.40	0.03-0.05	0.07	TRANSVERSE BAND	0.020	THIN-WALLED	TOTAL	
ACADIAE (SWALES, 1933)	0.45-0.62	0.15-0.21	0.033-0.039	0.022-0.025	POSTERIOR RING	0.019-0.020	THIN-WALLED	ENDS OF CAECA	
RHODANICUM CARRERE, 1936	0.63-0.80	0.28-0.38	0.044-0.058	0.060-0.072	POSTERIOR RING	0.021-0.022			
EROLAE YAMAGUTI, 1939	0.4-0.65	0.2-0.25	0.033-0.036	0.042-0.046	POSTERIOR RING	0.018-0.020	HEAVY-WALLED	TOTAL	
JAPONICUM YAMAGUTI, 1939	0.7-1.2	0.25-0.46	0.060-0.090	0.07-0.12	6-8 FOLLICLES	0.024-0.029	HEAVY-WALLED	ALMOST TO END	
URIAE YAMAGUTI, 1939	0.85-1.2	0.30-0.46	0.060-0.080	0.09-0.10	6-10 FOLLICLES	0.021-0.024	HEAVY-WALLED	ALMOST TO END	
CINCLI YAMAGUTI, 1939	0.96-1.2	0.42-0.48	0.093-0.10	0.18	ACINOUS	0.016-0.019	THIN-WALLED	ALMOST TO END	
OVATA RANKIN, 1939	0.40-0.52	0.19-0.30	0.03-0.04	0.03-0.05	POSTERIOR RING	0.019-0.024	THIN-WALLED	TO TESTES	
ARENARIA HADLEY&CASTLE, 1940	0.30-0.56	0.19-0.26	0.038-0.054	0.038-0.050	POSTERIOR RING	0.016-0.020	THIN-WALLED	TO OVARY	
CARIDINAE FROM SHIBUE, 1951	0.66	0.40	0.069×0.059	0.085	RING	0.020-0.026		TOTAL	
GLANDULOSA SP. NOV.	0.58	0.23	0.064	0.051	MEDIAN RING	0.022	THIN-WALLED	TOTAL	
PATULUS SP. NOV.	0.46	0.31	0.048	0.057	INCOMPLETE RING	0.021	THIN-WALLED	TOTAL	

vitellaria, it seems very likely that the structure of the vitellaria in some of the forms described by Yamaguti (1939) differs considerably (size and number of follicles) from that found in *M. glandulosa* and *M. patulus*. The extent of the cuticular spination and the nature of the vaginal wall appear to be clear-cut structural characters with little variation. In Etges' (1953) drawings depicting variation in the position of the vitellaria, cirrus-sac, and the testes, the ovary remains relatively stable. Furthermore, it is very probable that the disposition of the acetabulum and genital pore is rather uniform. It is for these reasons that a sketch of the relative arrangement of the genital pore, the acetabulum, and the ovary for each species is presented in Table I. It should be noted that figures were not available for some of the species.

From the statements above it is apparent that the members of this genus are very similar. This indicates either that they are of recent origin, or that they evolved some time ago and that few, if any, of the members have become extinct. Their lack of host specificity is a form of adaptability enabling them to persist even though one of their host species might become extinct. This path is closed to more specialized parasites. In any discussion of the relationships of these groups, the possibility of convergence should not be overlooked; however, the lack of knowledge of more stable characters presented by larval forms precludes the possibility of speculation along these lines.

Literature Cited

Baer, J. G. 1943 Les trématodes parasites de la musaraigne d'eau *Neomys fodiens* (Schreb.). Bull. Soc. Neuchâtel. Sci. Nat. **68**: 33–84.

Cable, R. M. and Kuns, M. L. 1951 The trematode family Microphallidae with the description of *Carnecphallus trilobatus* gen. et sp. nov., from Mexico. J. Parasit. **37**: 507–514.

Etges, F. J. 1953 Studies on the life histories of *Maritrema obstipum* (Van Cleave and Mueller, 1932) and *Levinseniella amnicolae* n. sp. (Trematoda: Microphallidae). J. Parasit. **39**: 643–662.

Hadley, C. E. and Castle, R. M. 1940 Description of a new species of *Maritrema* Nicoll, 1907, *Maritrema arenaria*, with studies of the life history. Biol. Bull. Woods Hole. **78**: 338–348.

Rausch, R. 1947 Some observations on the host relationships of *Microphallus opacus* (Ward, 1894) (Trematoda: Microphallidae). Tr. Am. Micro. Soc. **66**: 59–63.

Yamaguti, S. 1939 Studies on the helminth fauna of Japan. Part 25. Trematodes of Birds IV. Jap. J. Zool. **8**: 129–210.

AN UNUSUAL CASE OF SCHISTOSOMIASIS WITH SPECIAL EPIDEMIOLOGIC ASPECTS

EMMANUEL MARGOLIS
Hadassah University Hospital, Jerusalem, Israel

Schistosomiasis in Israel became a serious medical and epidemiological problem with the recent immigration of thousands of people from Yemen, Iraq and other eastern countries. The highest incidence of positive response to the skin test for schistosomiasis was found among immigrants who arrived from Yemen during the last five or six years. Of 690 immigrants from Yemen, 38 percent of the males and 30 percent of the females reacted positively; the percentage of "positive reactors" was directly proportional to age and varied between 21 in children 3–4 years old and 85 in adults over 50 (Eliakim and Davies, 1954).

The following case faces some of the characteristic medical and epidemiological problems; it concerns a boy, 18 years old, born in Yemen, the fifth son of a healthy family, living in Israel for 5 years. It is to be stressed that the boy could not recall any illness with diarrhoea, nor other gastro-intestinal disturbances and, apparently, did not acquire infection in Israel.

The present illness began, six months before his first visit to our clinic, with vague abdominal discomfort lasting two months. Then there appeared diarrhoea with bloody and sometimes purulent stools. He had 4 to 10 bowel movements daily and felt spontaneous abdominal pain particularly along the left side of the abdomen. He lost 5 kg. during this period, felt very ill, and was unable to carry on his daily work.

Physical examination revealed a normally developed youth in a fair state of nutrition. His tongue was coated and his breath was foul. The liver was enlarged 4 cm. below the costal margin and the spleen some 6 cm. A peculiar finding was gross clubbing of fingers and toes. The patient stated that, so far as he remembered, his fingers and toes were always so shaped.

Laboratory findings: Erythrocyte sedimentation rate—90/120 after Westergren, red cells 4.5 million per cmm., hemoglobin 10.0 g. percent, white cells 8000 per cmm.; the differential count showed 12 eosinophiles percent. There was a slight decrease in the total proteins of the serum, the values being 2.6 g. percent albumen and 2.6 g. percent globulin. Liver function tests, including formol gel, were normal. Nothing abnormal was found in the urine. Ordinary microscopic examination of feces revealed numerous eggs of *Schistosoma mansoni*. On proctoscopy five large polyps with ulceration were seen in the rectum.

DISCUSSION

One of the earliest reports on Schistosomiasis in Israel, then Palestine, was made by Searle (1920); the report is concerned with *Schistosoma haematobium* only. Searle stressed that he never found the ova of *Schistosoma mansoni* nor did he find the intermediary host of this species—the Planorbis. At that time, *Schistosoma haematobium* was thought to be found only in the country, until Malchi (1924) described two cases of *Schistosoma mansoni* infection, contracted probably in Yemen. However new cases were always rare and for a long time no epi-

Received for publication, May 9, 1955.

demic had been recorded in Israel. During the years 1949–1952 only 19 new cases of *Schistosoma mansoni* infection were reported (Frankl, 1953). The main explanation for this phenomenon, according to Witenberg and Saliternik (1955), lies in the fact that the distribution of the snail-vector of *Schistosoma mansoni*, the Planorbis, is limited to a small section of Yarkon River.

The infection of *Planorbis alexandriensis* by the Yemenite strain of *Schistosoma mansoni* seems to occur in nature but very rarely (Eliakim and Davies, 1954). The problem whether the transmission of the Yemenite strain of *Schistosoma mansoni* through the intermediary host found in the Yarkon is possible is now being studied by Witenberg (personal communication). If the studies confirm this possibility the problem of the longevity of *Schistosoma mansoni* attains special importance. Most of the new immigrants into Israel, although reacting positively to the skin test, are free from clinical signs; they recovered from the acute stage of the disease many years ago, when in their original countries. Among these clinically silent positive reactors there are, probably, many egg carriers, who, through the intermediary host in the Yarkon (if it is proved to be susceptible), could become dangerous spreaders of the disease and then a new epidemic focus of *Schistosoma mansoni* could develop. As stressed by Meleney (1954) movement of troops during military operations, migrations of peoples, improvement in transportation, and the expansion of irrigation systems have all been responsible for extension of the infection where suitable hosts are present. The recent report by Witenberg and Saliternik (1955), of urinary Schistosomiasis in a place not hitherto known to harbor *Bulinus contortus*, where almost 50 percent of children and youths (all born in Israel) proved to be infected, again confirms the statement of Meleney and should serve as a serious warning.

In our case it has been established that the patient had never bathed in nor had other contact with the waters of the Yarkon, which, as mentioned above, is known to be (Witenberg and Saliternik, 1955), at least for the time being, the only harbor of the intermediary host, namely *Planorbis alexandriensis*. Hence it can be deduced that he probably did not acquire the disease in Israel. It is therefore concluded that we are confronted with an old chronic case, which flared up for reasons unknown. The acute stage of the disease could have occurred many years before and could have passed silently as the patient does not recall even a single symptom to mark the onset and the course of the disease. Hoff (1936) reported two cases of schistosomiasis; in the first case absence of the usual dysenteric symptoms (as in the present case) caused the condition to be overlooked for many years. After the initial warning signals the existence of schistosomiasis may be, as the author pointed out, entirely compatible with perfect health until damage to the liver initiates the secondary phase, that of cirrhosis. In the literature cases are reported which show an even greater longevity of the parasite. Berberian, Paquin and Fantauzzi (1953) described the case of a Yemenite, who 26 years after immigration into the United States and possibly 40 years after infection, was still passing the ova of both *Schistosoma* species in stools and urine. In their case also the typical signs and symptoms of both infections had been silent, indicating, as the authors state, that, if initial infection is not heavy and reinfection does not occur, specific symptoms of the disease may not appear. Wallerstein (1946) reported a case of *Schistosoma mansoni* infection in a Puerto Rican woman complicated with

a precancerous polyp 12.5 cm. from the anus. It was evident from the history that the infection was acquired not less than 26 years before the chance discovery of the disease.

The patient's clubbing is a question of interest as well. It is difficult to assume that this is a case of hereditary clubbing as described by Mendlowitz (1942) and by Davis (1946), because the family examination revealed no clubbing. It is probable that the schistosomiasis, whose onset in his early childhood the patient cannot recall, is connected with this clubbing. No evidence of pulmonary or heart disease, the commonest predecessors of acquired clubbing, was found; while it was established through the history, the laboratory, proctoscopic, and epidemiologic data, that he must have harbored the infection for a long time. On the other hand review of more recent published reports made by Lipman and Massie (1952, p. 210) indicates that 5 to 10 percent of cases with clubbing are associated with the gastrointestinal tract, including the liver. Of 10 cases of ulcerative colitis two were found by Branwood (1949) to have clubbing of the fingers. He cited French authors describing clubbed fingers in cases of polyposis of the colon and chronic amebiasis. In his own series one patient, who had marked finger clubbing, gave a history of both amebic and bacillary dysentery and was found to be suffering from chronic amebiasis.

Our patient was given the following treatment: for five successive days an i.v. injection of 2 percent potassium antimony tartrate, the initial dose being 40 mg. with 20 mg. added every day up to 120 mg. given in the fifth day. From then on a maintenance dose of 100 mg. every second day was given up to a total of 1800 mg. Cure was not achieved, as examination of the feces again revealed the eggs of *Schistosoma mansoni*. Since side effects were not seen, we decided after an interval of only six weeks to treat again using the same schedule. This time the results were good. No *Schistosoma mansoni* eggs were found in repeated examination of the stools and the clinical picture improved considerably. The red cell count reached 4.7 million and the hemoglobin increased to 10.5 g. percent. A significant improvement of the erythrocyte sedimentation rate was noticed, 14/51 as compared with 90/120 previously. The eosinophiles went down to 7 percent.

The patient is now awaiting surgical removal of the rectal polyps. This intervention is expressly indicated; the lesions, while benign in themselves, can be precursors of malignant change. As stated by Karsner (1946, p. 327), "In man, disease caused by higher forms of animal parasites may result in cancer, notably Schistosomiasis. *Schistosoma hematobium* infestations may cause papilloma of the bladder, sometimes with subsequent carcinoma; this may also happen in the rectum." In the case reported by Wallerstein (1949) a pedunculated polyp, observed 12.5 cm. from the anus, was removed and on biopsy was diagnosed as precancerous. The polyp also contained many eggs of *Schistosoma mansoni*, but repeated stool examinations were negative for ova. Fairley (1931) cited a case of vesical Schistosomiasis complicated by widespread carcinomatosis of the bladder. The urine of this patient contained many eggs of *Schistosoma haematobium*.

SUMMARY

A case of *Schistosoma mansoni* infection is reported in a patient aged 18. The infection was undoubtedly acquired in early childhood in Yemen. Typical signs

and symptoms have been absent throughout this long period until a flare up occurred for reasons unknown. The patient showed severe clubbing of fingers and toes which could not be related to any pulmonary or heart disease and was probably associated with the chronic intestinal illness of the patient. The patient received two courses of potassium antimony tartrate with striking improvement after the second course. Polyps were found on rectoscopy and the question of possible malignant change is considered. The problem of the transmission of the Yemenite strain of *Schistosoma mansoni* through the intermediary host found in the Yarkon River in Israel is mentioned. In this connection the importance of the problem of the longevity of *Schistosoma* is discussed.

LITERATURE CITED

BERBERIAN, D. A., PAQUIN, H. O. AND FANTAUZZI, A. 1953 Longevity of *Schistosoma haematobium* and *Schistosoma mansoni:* observations based on a case. J. Parasit. **39:** 517–519.

BRANWOOD, A. W. 1949 Clubbing of fingers. Clin. J. **78:** 167–179.

DAVIS, E. 1946 Hereditary clubbing of digits in 2 families. Brit. Med. J. **1:** 128–129.

ELIAKIM, M. AND DAVIES, A. M. 1954 The Incidence of bilharziasis in immigrants from Yemen, Iraq, Morocco and Iran. (In Hebrew) Harefuah-J. Med. Ass. Israel **47:** 121–124.

FAIRLEY, N. H. 1931 Vesical schistosomiasis complicated by carcinoma. Brit. Med. J. **2:** 983–985.

FRANKL, O. 1953 Early intestinal schistosomiasis. (In Hebrew) Harefuah-J. Med. Ass. Israel **45:** 83–85.

HOFF, A. 1936 Schistosomiasis-report of two cases. J. Am. Med. Ass. **107:** 1375–1378.

KARSNER, H. T. 1949 Human Pathology, 7th Edition, Philadelphia, Lippincott, 927 pp.

LIPMAN, B. S. AND MASSIE, E. 1952 Clubbed fingers and hypertrophic osteoarthropathy. In Macbryde, C. M. (Ed.) "Signs and Symptoms," 2 Edition, Philadelphia, Lippincott, 783 pp.

MALCHI, A. 1924 Two cases of schistosomiasis mansoni in Palestine. (In Hebrew) Harefuah-J. Med. Ass. Israel **1:** 75–79.

MELENEY, H. E. 1954 Problems in the control of schistosomiasis. Am. J. Trop. Med. and Hyg. **3:** 209–218.

MENDLOWITZ, M. 1942 Clubbing and hypertrophic osteoarthropathy. Medicine **21:** 269–306.

SEARLE, C. 1920 Bilharzia and malaria during the Palestine campaign. J. Roy. Army M. Corps. **34:** 15–34.

WALLERSTEIN, R. S. 1949 Longevity of *Schistosoma mansoni:* observation based on a case. Am. J. Trop. Med. **29:** 717–722.

WITENBERG, G. AND SALITERNIK, Z. 1955 Danger of scistosomiasis in Israel. (In Hebrew) Harefuah-J. Med. Ass. Israel **48:** 219–220.

THE EFFECT OF DIET ON THE LENGTH OF THIRD STAGE LARVAE PRODUCED BY ADULT *HAEMONCHUS CONTORTUS* HARBORED BY LAMBS[1]

R. F. SHUMARD,[2] C. A. HERRICK AND A. L. POPE
The University of Wisconsin

The life cycle of *Haemonchus contortus*, as described by Ransom (1906) and Veglia (1915), contains two free-living larval stages. During these stages the larvae presumably feed on microorganisms and debris contained in the fecal matter of the hosts of their parents. Richard, *et al.* (1954) showed that additions of cobalt to the diet of lambs infected with stomach worms increased egg production of the female worms and enabled them to produce eggs at an earlier time following infection. Since this mineral had such an effect on the immature and adult female *Haemonchus*, it was presumed that cobalt, other minerals, or the products from the minerals found in the feces of the lamb host might also affect the growth of the larvae.

MATERIALS AND METHODS

Sixteen parasite-free lambs were allotted to four lots, four lambs per lot. All were fed a basal ration consisting of alfalfa-grass hay, oat straw and ground corn. Lot I was given iodized salt; Lot II, cobaltized salt,[3] Lot III, salt plus manganese[4] and Lot IV, trace mineralized salt.[5] All male lambs had been castrated and all lambs were docked.

Three lambs in each lot were given approximately 50,000 infective *Haemonchus contortus* larvae by means of a drenching syringe. The fourth lamb in each lot was designated a control animal and the feces of these animals were examined frequently for helminth ova. They were consistently found to be free of ova except for a few *Strongyloides* sp. Twenty-seven to 30 days following the administration of the larvae, feces were collected from all of the lambs. The feces of each of the control lambs was crushed by hand, mixed with charcoal and placed, several millimeters thick, on two thicknesses of moistened filter paper in Petri dishes. The feces from each of the infected lambs were crushed and placed in saturated sodium chloride solution in large centrifuge tubes. With the aid of centrifugation the eggs were separated from the fecal material and were gathered from the surface of the solution. The eggs were then washed once with 0.9 percent sodium chloride solution and three times with tap water. Aliquots of the resulting samples were removed and the number of eggs counted. Approximately 8,000 fertilized eggs were placed on each control lamb's feces in the Petri dishes. The scheme for culturing was as follows:

Received for publication, April 27, 1955.
[1] This research was supported by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.
[2] Present address: Department of Veterinary Science, North Dakota Agricultural Experiment Station, Fargo, North Dakota.
From the Departments of Veterinary Science and Animal Husbandry, University of Wisconsin paper No. N.S. 163.
[3] 6.72 grams $CoSO_4$ in 100 lbs. salt (iodized).
[4] 100.50 grams $MnSO_4 \cdot H_2O$ in 100 lbs. salt (iodized).
[5] The following in 100 lbs. salt: 10.25 grams I, 5.00 grams $CoCo_3$, 50.25 grams $CuSO_4$, 50.25 grams Fe_2O_3, 100.50 grams $MnSO_4 \cdot H_2O$, 102.50 grams Na_2SO_4, 205.00 grams $Na_2S_2O_3$, 361.80 grams $FeSO_4$.

Eggs from the feces of infected lambs in Lot I (lambs 510, 543 and 548 respectively) were placed on the fecal material from control lamb 525 (Lot I), from control lamb 531 (Lot II), from control lamb 505 (Lot III), and from control lamb 527 (Lot IV). Eggs from the feces of infected lambs in Lots II, III, and IV were similarly treated; eggs from each of the infected lambs being placed in the feces of control lambs from each of the four lots.

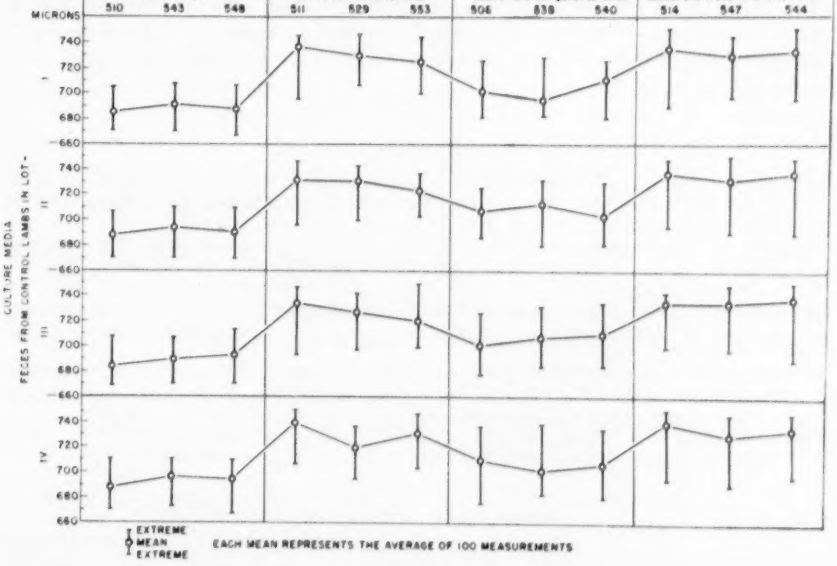
All cultures were kept open and moist, at least three inches from each other, in a dark chamber at room temperature. After seven days, the larvae were collected from the cultures by means of a Baermann Funnel (Cort, *et al.*, 1922). An aliquot of the larval suspension was taken, placed on a microscope slide, covered with a cover slip, and then placed on an electric hot plate for five seconds. The heat killed the larvae and also caused the majority of them to straighten so that they could be measured. One hundred larvae from each culture were measured. Larvae that contained granular instead of sharply defined intestinal cells, or that had a highly wrinkled sheath, or that possessed no sheath, were not measured. The tail portion of the sheath from the tip of the tail of the third stage larvae posteriorly, was not measured.

RESULTS AND DISCUSSION

Dikmans and Andrews (1933) found that the infective larvae of *Haemonchus contortus* measured from 650 to 751 microns in length, including the sheath. The sheath portion of the tail was found to extend from 65 to 78 microns beyond the end of the larva. The infective larvae, not including the tail portion of the sheath, would therefore vary from 585 to 673 microns in length if we assume that the tail portion of the sheath was proportional to the total length of the larva. In the

Figure 1

Measurements of larvae cultured (from eggs from the feces of infected lambs in the four nutritional lots) in the feces of control lambs in the four nutritional lots.


Infective larvae cultured from eggs in feces of Lot I (basal) lambs
510 543 548
Infective larvae cultured from eggs in feces of Lot II (basal and cobalt) lambs
511 529 553
Infective larvae cultured from eggs in feces of Lot III (basal and manganese) lambs
506 538 540
Infective larvae cultured from eggs in feces of Lot IV (basal and trace minerals) lambs
514 547 544
MICRONS
740
720
700
680
660
CULTURE MEDIA
FECES FROM CONTROL LAMBS IN LOT-
I
II
III
IV
EXTREME
MEAN
EXTREME
EACH MEAN REPRESENTS THE AVERAGE OF 100 MEASUREMENTS

present study, the third stage larvae varied from 666 to 750 microns in length, not including the tail portion of the sheath.

Dikmans and Andrews used physiological saline and warm Fleming solution to kill the larvae. In this study, the larvae were heat-killed in tap water. This difference in methods may account for some of the variation obtained. Dikmans and Andrews did not state the nutrition of the host animals from which the nematodes, used as sources of eggs, were collected; but did state that they were obtained from abattoirs at Beltsville, Maryland, and in the vicinity of Washington, D. C. The nutrition of the hosts in this study was known and controlled.

The means and extremes of the measurements are shown in Figure I. It is evident that no noteworthy difference in length of third-stage larvae was obtained when the eggs removed from the feces of any of the four nutritional lots were cultured in the feces of a control lamb from other nutritional lots. The length of the larvae from any one of the nutritional lots remained essentially the same regardless of which fecal material was used as a culture medium. Analysis of variance showed a highly significant difference among the lengths of the larvae that was dependent on the mineral diet being received by the host of the parents of the larvae. It seems, therefore, that the size of the third-stage larvae was not dependent on the nutrition of the two free-living larval stages but rather on the nutrition of the parents of the larvae and, therefore, on the nutrition of the host.

SUMMARY

Eggs from pure infections of *Haemonchus contortus* from lambs receiving basal rations plus iodized salt, cobaltized salt, salt with manganese, or trace mineralized salt were cultured in the feces of uninfected lambs on the same rations. The contents of the culture media had no demonstrable effect on the growth of the larvae of this nematode as determined by measurement of the third stage. Highly significant differences in length of the larvae were demonstrated and were apparently due to the nutrition of the host harboring the parents of the larvae. Lambs receiving cobaltized salt or trace mineralized salt harbored adults that produced eggs from which the longer larvae developed.

REFERENCES CITED

CORT, W. W., ACKERT, J. E., AUGUSTINE, D. L., AND PAYNE, F. K. 1922 Investigations on the control of hookworm disease. The description of an apparatus for isolating infective hookworm larvae from soil. Am. J. Hyg. **2:** 1–16.

DIKMANS, G. AND ANDREWS, J. S. 1933 A comparative morphological study of the infective larvae of the common nematodes parasites in the alimentary tract of sheep. Tr. Am. Micr. Soc. **52:** 1–25.

RANSOM, B. H. 1906 The life history of the twisted wire worm (*Haemonchus contortus*) of sheep and other ruminants. (Preliminary report) Circ. 93, Bureau Animal Industry, U. S. Dept. Agric. pp. 1-7.

RICHARD, R. M., SHUMARD, R. F., POPE, A. L., PHILLIPS, P. H., HERRICK C. A., AND BOHSTEDT, G. 1954 The effect of certain mineral supplements on lambs infected with the stomach worm (*Haemonchus contortus*). J. Animal Sci. **13:** 694–705.

VEGLIA, F. 1915 The anatomy and life history of *Haemonchus contortus* (Rud.) 3. and 4. Rep. Dir. Vet. Research, Dept. Agri. Union So. Africa. pp. 347–500.

TOXOPLASMA IN TISSUES OF MAN AND PETS

A. C. KRAUSE

Division of Opthalmology, Department of Surgury, University of Chicago, Chicago, Illinois

In animals experimental infection with *Toxoplasma gondii* often lasts for years. In man ocular toxoplasmosis may at times become reactivated years after the initial lesion is seen. This suggests that a dormant infection is present. It therefore seems that a chronic cryptic infection in animals and man is quite common without any obvious evience of toxoplasmosis.

Since man and his household pets such as cats and dogs commonly harbor *Toxoplasma gondii* according to the serological evidence obtained from the Sabin-Feldman tests it was believed that the organism perhaps could be isolated in possible dormant infection by inoculation of tissue into susceptible mice. The purpose of this paper is to report on the infections which were found with this procedure.

PROCEDURE

The method employed to isolate the *Toxoplasma gondii* from animals was similar to that used by Eyles (1952). A group of 23 full grown dogs that were rejected as house pets in Chicago were brought to the laboratory and killed by electrocution. Seven cats over two years old and three kittens from three to six months of age that were pets in the city and on the farm were killed with ether. The dissection of the tissues was carried out immediately in an aseptic manner.

An effort was made to isolate *Toxoplasma gondii* from the animals by inoculating white mice intraperitoneally with 0.5 cc. of a suspension of the tissue made by grinding five grams of tissue aseptically in 10 cc. of physiological salt solution with a mortar and pestle. The liver and brain and occasionally the spleen and lung were each injected into a group of three disease-free white mice that were raised in an inbred colony for this purpose. All mice were kept in individual cages away from other stock animals. The best method for obtaining samples of brain from animals with thick bony skulls was by means of a circular cup saw for cutting metal discs that was attached to an electrical hand drill.

After inoculation the mice were watched daily for four weeks. The surviving animals were killed with ether and aseptically autopsied. Smears were made from the peritoneal fluid, liver, lung, brain, and occasionally the spleen, of dead and surviving animals. They were stained with the Giemsa stain for parasites. Over 200 microscopic fields under oil immersion at 970 magnifications were searched for *Toxoplasma gondii*. The lungs, liver and brain of the sacrificed mice were pooled according to original tissue and were re-inoculated into groups of three mice. This procedure was repeated through three periods of four weeks. The tissues of the dying mice with questionable toxoplasmic infection were followed after injection in separate groups of nine mice. A smear of the washing of peritoneum by saline besides the routine smears of the organs was made in the terminal group of mice. The death rate from disease was remarkably low in the isolated colony of mice.

Tissues were also obtained from the postmortem examination of thirty patients

Received for publication, May 4, 1955.

A grant-in-aid from the National Council to Combat Blindness, Inc., and the Douglas Smith Foundation has made this study possible.

usually on the same day they died and a few the following morning after death. About five or more grams of heart, spleen and brain tissue were removed aseptically. In five patients samples of cortical tissue of the brain were also taken. The patients had died from chronic degenerative and infectious disease and from cancer. The distribution in age was: five from six months to ten years of age, eleven from ten to 50 years and 14 over 50 years of age. These tissues were passaged singly in groups of mice in the same manner as those of the dogs and cats. If there were any doubt about toxoplasmic infection of the inoculated mice, nine other mice were injected and followed for evidence of infection, particularly in the liver and peritoneal fluid. There was no evidence of *Toxoplasma gondii* in the human tissues although the pathologist was well acquainted with the appearance of the organism in standard stained tissue.

RESULTS

Twenty-three dogs were tested for toxoplasmic infection by inoculation of selected tissue into mice. Only in one full grown mongrel dog which was a city house pet was *Toxoplasma gondii* found in the brain on the third passage into sub-inoculated mice. The *Toxoplasma gondii* were seen in the liver and peritoneal fluid smears of the mice which died seven to ten days after the inoculation. It was believed that the strain of *Toxoplasma gondii* was only mildly virulent. No evidence of infection was obtained in the ten healthy house cats tested in an identical manner. In the brain, liver, heart and spleen of 30 patients no *Toxoplasma gondii* were obtained through passage in mice.

DISCUSSION

No doubt exists about the persistency of experimental toxoplasmic infection in animals. A common method of keeping a stock of living *Toxoplasma gondii* on hand for years in this laboratory is by inoculation of white rats. The organism tends to infect the brain for at least two or more years. Ruchman and Fowler (1951) found these parasites in the brain of rats two years after inoculation. Jacobs and Melton (1952) mention that a pigeon carried the organism for 33 months in the brain.

Toxoplasmic infection is evidently commonly cryptic and chronic in man and his animal pets. Feldman (1953) by laboratory tests observed that in a group of cats 34 percent and in a group of dogs 59 percent gave positive reactions. Various groups of humans ranged from none to 65 percent in positive tests. These reactions suggest long term infections with *Toxoplasma gondii*.

The mothers giving birth to infants with acute toxoplasmosis apparently carry *Toxoplasma gondii* seemingly as a latent chronic infection without having clinical symptoms. Numerous patients with chorioretinitis have given reasonably high titers with the dye test. In the University of Chicago Eye Clinic two female adolescents with an active uveitis gave four plus reactions with the rabbit serum protective test in 1942. Every few years the uveitis reoccurred and the toxoplasmin reactions were positive. New lesions formed adjacent to the old scars. The last flare up was in 1955. It is probable that the liberation of the parasites in the pseudocysts near the old scars resulted in a new inflammation. The isolation of the parasite from the eye with uveitis by Jacobs, Fair and Bickerton (1954) is further evidence of chronic infection in man.

Organisms that compare well with *Toxoplasma gondii* have been found in sections of the tissues of dogs and cats by Wickham and Carne (1950) and of cats by Olafson and Monlux (1942). In reviewed post-mortem examinations of patients the organisms were observed in heart and brain tissue by Plaut (1946), Kean and Grocott (1947) and Callahan, Russell and Smith (1946).

It seems reasonable to believe the *Toxoplasma gondii* lies dormant in many domestic pets and man. The high percentage of the dye titer test in relatively unselected individuals leads one to surmise that the organism should be demonstrated by passage through mice, perhaps once or twice out of three individuals. However, in man, Callahan, Russell and Smith (1946) discovered parasites similar in appearance to *Toxoplasma gondii* in tissues from five autopsies of 10,000 patients. In this search for organisms the tissues were not specifically prepared for this examination. This is a low ratio of 1 to 2000 of infection detectable by section as compared with a dye titer of over 50 percent in man.

Although only one dog was found infected with *Toxplasma gondii* out of 23 dogs and no evidence of infection was obtained in ten cats and 30 humans, the results give rise to certain inferences. The specimen of tissue was very small in relation to the size of the organ and the parasites may have been missed. Furthermore the selection of tissue was limited and other tissues may be infected. This is true also in living patients. In a few patients with chorioretinitis that gave marked skin reactions to toxoplasmin, bone marrow from the sternum and liver from biopsies that were injected intracerebrally in mice gave no evidence of organisms. There is also a possibility of the death of the organism in the tissues after autopsy. The tissues from the animals were used a few minutes after death but in tissue from patients there was an unavoidable loss of time for the autopsy. It is unlikely that a strain of non-virulent organism passaged through mice would be missed. Of course a few organisms in the original inoculum or a non-virulent strain might be destroyed in mice. This may not be a factor because the inbred mice in the laboratory were easily infected by three different strains of *Toxoplasma gondii* obtained elsewhere from patients.

It is very probable that if the *Toxoplasma gondii* survive in a chronic infection of long duration the parasites are dormant in cysts in or near the old lesion or are inactive in a few organs such as the heart, liver or brain. The total number of organisms and their foci may be relatively few.

SUMMARY

Suspension of the brain, liver and occasionally lung and spleen of 23 dogs and ten cats that were house and farm pets were inoculated into white mice. One strain of *Toxoplasma gondii* was obtained from one adult dog. The tissue from the liver, heart and spleen from the post-mortem examination of 30 patients and the brain tissue from ten patients gave no evidence for infection when they were treated in a similar manner.

Random sampling of tissue for *Toxoplasma gondii* from dogs, cats and man by passage of the parasite through mice reveals slight or no evidence of chronic infection. The method may be applied better to smaller animals of which the whole organs can be used.

REFERENCES CITED

CALLAHAN, W. P. JR., RUSSELL, W. O. AND SMITH, M. G. 1946 Medicine **25**: 343–397.

EYLES, D. E. 1952 Toxoplasma in the Norway rat. J. Parasitol. **38**: 226–229.

FELDMAN, H. A. 1953 The clinical manifestations and laboratory diagnosis of toxoplasmosis. Amer. J. Trop. Med. & Hyg. **2**: 420–428.

JACOBS, L., FAIR, J. R. AND BICKERTON, J. H. 1954 Adult ocular toxoplasmosis. A preliminary report of a parasitologically proved case. Arch. Ophth. **51**: 287.

——— AND MELTON, M. L. 1952 Observations on toxoplasmosis in pigeons. J. Parasitol. **38**: (4, sect. 2.) 12.

KEAN, B. H. AND GROCOTT, R. C. 1947 Asymptomatic toxoplasmosis. Amer. J. Trop. Med. **27**: 745–748.

OLAFSON, P. AND MONLUX, W. S. 1942 Toxoplasma infections in animals. Cornell Vet. **32**: 16–190.

PLAUT, A. 1946 The problem of human toxoplasma carriers. Amer. J. Path. **22**: 427–430.

RUCHMAN, I. AND JOHANSMANN, R. J. 1951 Biological properties of a strain of *Toxoplasma* recovered from a fatal case of congenital toxoplasmosis. Amer. J. Trop. Med. **28**: 687–695.

WICKHAM, N. AND CARNE, H. R. 1950 Toxoplasmosis in domestic animals in Australia. Austral. Vet. J. **26**: 1–3.

PHYSICAL OBSERVATIONS ON *ECHINOCOCCUS* EGGS, PART I

H. F. MEYERS*

The methods of transmission of hydatid diseases, its effects on various intermediate and final hosts and its detection, both clinically and physically, have been rather thoroughly investigated (Dew, 1928). However, the physical and chemical properties of the *Echinococcus* egg have received little consideration. One of the principal obstructions has been the lack of suitable experimental animals which could be obtained at moderate cost. The observation by Rausch and Schiller (1951) that the microtine rodents serve as intermediate hosts for a species of *Echinococcus* has alleviated this problem of experimental animals in Alaska. The purpose of this paper is to present several physical observations made on *Echinococcus sibiricensis* Rausch and Schiller, 1954, using the red-backed vole *Clethrionomys rutilus dawsoni* (Merriam) as a laboratory animal. Work on the chemical resistance of *Echinococcus* eggs is anticipated by the writer, hence the necessity for determining weight and density was indicated.

The carcasses of arctic foxes, one of the final hosts for *Echinococcus sibiricensis*, were collected and maintained in a frozen condition. When eggs were needed for an experiment a fox was autopsied, and the work (see procedure) concluded the same day. Three red-backed voles were infected with eggs from each fox to determine viability. Foxes were kept from four to six weeks at -26° C. in a deep freeze without any apparent damage to the eggs, since they were viable in all instances. Work on the resistance of *Echinococcus* eggs to low temperature has recently been completed by Schiller (Ms.).

Since the *Echinococcus* eggs are elliptical, almost spherical, it was assumed that they would follow Stokes' law reasonably well. Essentially, this law states that when a small sphere falls under the action of gravity through a viscous medium it

Received for publication, May 6, 1955.

* Chemist, Arctic Health Research Center, Public Health Service, Department of Health, Education, and Welfare, Anchorage, Alaska.

ultimately acquires a constant velocity. To the author's knowledge Stokes' law has not heretofore been used to determine weight or density of cestode eggs. In order to utilize Stokes' law under microscopic conditions, it was found convenient to employ a Sedgwick-Rafter counting cell as the falling-chamber. A chemical microscope with rotating stage which could be tilted 90° was used for observing the falling eggs. By rotating the stage 180°, the eggs could be made to pass the field of view repeatedly. A one millimeter Whipple micrometer disc was used in the eyepiece and the time required for an egg to fall this unit distance was tabulated. Freshly boiled and cooled distilled water was used as the liquid medium. It was assumed that very little osmosis, if any, would take place during the period of time required for a series of tabulations, usually about 1.5 hours.

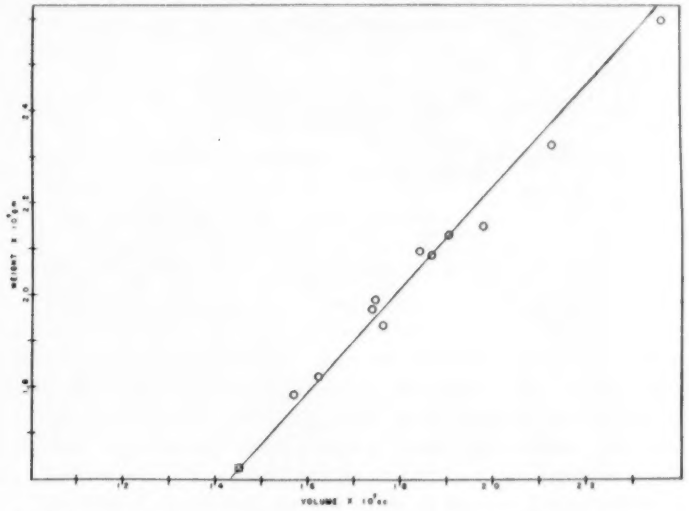


FIG. 1. Relation of weight to volume in eggs of *Echinococcus sibiricensis*.

PROCEDURE

Foxes were removed from the deep freeze, allowed to thaw at room temperature, autopsied and the intestinal content collected in a large dish. Extraneous material placed on a microscope slide with a drop of water. The gravid segments were ruptured with a needle and the water containing the eggs was taken up in a capillary pipette. The water and eggs were transferred to a Sedgwick-Rafter counting chamber, the cover slip applied and the cell filled with distilled water. The cell was secured to the microscope stage with a rubber band, thus allowing several centimeters of movement in two directions with a mechanical stage. After the microscope was tilted 90° the heavier debris was allowed to settle before time readings were taken on the eggs. This was done to prevent currents from interfering with the natural fall of the eggs. Up to one hundred readings were taken for each sample was removed by washing with tap water, and cestodes which had settled were removed with a rubber bulb pipette. Two to four gravid worms were selected and (time per unit distance), the microscope stage righted and an average diameter for

the eggs determined using an ocular micrometer scale. Both length and width were averaged together on 30 to 50 eggs in all cases.

DISCUSSION

Stokes' law $r = \sqrt{\frac{9/2\,\eta\,\frac{dx/dt}{(d_p - d_m)g}}}$ when rearranged gives $d_p = d_m + \frac{9/2\ dx/dt}{g\ r^2}$ where d_p and d_m are density of particle and medium respectively in g/cm.^{3}, dx/dt is velocity in cm./sec., η is coefficient of viscosity in dyne-seconds per cm.^{2}, g is gravity in cm./sec.^{2}, and r is radius of particle in cm. Number one in the table was calculated as follows:

$d_m = .998203$

$dx/dt = 6.85 \times 10^{-3}$ cm./sec.

$\eta = 10.05 \times 10^{-3}$ dyne-seconds/cm.^{2}

$g = 982.009$ cm./sec.^{2} (Lat. 61° 14′)

$r = 1.57 \times 10^{-3}$ cm.

$$d_p = .998203 + \frac{9/2 \times 6.85 \times 10^{-3} \times 10.05 \times 10^{-3}}{982.009 \times (1.57 \times 10^{-3})^2}$$

$$d_p = .998203 + \frac{6.204 \times 10^{-4}}{48.590 \times 10^{-4}} = /.998203 + .1276$$

$d_p = 1.1258$

$V_p = 4/3\ r^3 = 4/3 \times 3.1416 \times 3.892 \times 10^{-9} = 16.29 \times 10^{-9}$ cc

$wt_p = d_p \times V_p = 1.1258 \times 16.29 \times 10^{-9} = 18.34 \times 10^{-9}$ g.

Figures for the above determination were calculated at 20° C.

Since the Sedgwick-Rafter counting cell is not adaptable to the use of thermocouples, rigid control of temperature was not practical. During the course of time required to take a series of readings the room temperature varied from two to three degrees centigrade. It is therefore reasonable to assume that the temperature changes were at least as great in the falling chamber. The room temperature was taken every five minutes and these readings were averaged for the experiment. While it was assumed that no adhesive or repulsing forces were present, eggs were observed in clusters of two or three in several instances. These were naturally disregarded.

The pertinent data on 12 separate determinations are shown in Table I. Velocity and radius were measured. Volume was calculated from the radius, and weight was calculated from the rate of fall. Weight was plotted against volume (see graph), and the curve was drawn with slope 1.1184 originating at zero. The devia-

TABLE I.—*Experimental Results*

	$dx/dt \times 10^3$ cm./sec.	Radius cm. $\times 10^3$	Temp. °C.	Volume cc $\times 10^9$	Weight g $\times 10^9$	Density	Deviation of density from mean
1.	6.85	1.57	20.0	16.29	18.34	1.1258	.0073
2.	5.60	1.61	19.5	17.60	19.34	1.0986	.0198
3.	5.38	1.67	19.6	19.78	21.51	1.0872	.0311
4.	6.58	1.51	22.5	14.50	16.28	1.1226	.0042
5.	7.74	1.60	20.7	17.37	19.70	1.1338	.0154
6.	6.33	1.71	20.7	21.25	23.28	1.0952	.0231
7.	7.24	1.65	20.1	19.05	21.33	1.1194	.0010
8.	8.67	1.63	22.0	18.40	20.97	1.1396	.0212
9.	7.49	1.55	21.0	15.68	17.84	1.1375	.0191
10.	7.99	1.60	19.5	17.44	19.91	1.1412	.0227
11.	7.40	1.64	21.6	18.67	20.89	1.1189	.0005
12.	6.71	1.78	18.2	23.61	25.99	1.1006	.0177
Average				18.30	20.44	1.1184	.0153

tion of the points is well within the limits of experimental error. In conclusion, since weight and volume are directly proportional, weight can be ascertained simply by multiplying $4/3\ \pi\ r^3$ by the density given.

ACKNOWLEDGMENT

The writer is sincerely grateful to Dr. Robert Rausch and Mr. Everett L. Schiller, Arctic Health Research Center, for their help and cooperation in acquiring experimental material and for suggestions in handling it.

REFERENCES

DEW, H. R. 1928 Hydatid disease, its pathology, diagnosis and treatment. Aust. Medical Pub. Co. Sydney, 429 pp

RAUSCH, R. AND SCHILLER, E. L. 1951 Hydatid disease (*Echinococcosis*) in Alaska and the importance of rodent intermediate hosts. Science **113** (2925): 57–58.

RESEARCH NOTES

A NEW HOST RECORD FOR *TRYPANOSOMA AMBYSTOMAE* WITH THE DESCRIPTION OF AN ADDITIONAL MORPHOLOGICAL TYPE OF THE PARASITE

Following the description of *Trypanosoma ambystomae* from *Ambystoma gracile* (Lehmann, 1954, J. Parasit. **40**: 656–659) the writer has examined the blood of some other western Oregon Amphibia as follows: *Ambystoma gracile* (6), *Dicamptodon ensatus* (2), *Hyla regilla* (8), *Rana a. aurora* (3) and *Taricha granulosa* (17).

T. ambystomae was found in the blood of all larval *Ambystoma gracile* from Owl Creek, Linn County and, additionally, a haemoflagellate was recovered from a single *Taricha granulosa* taken from the same location; neither *T. granulosa* from other areas nor the other species of Amphibia were infected.

Observations of Wright-stained, thin smears showed that the flagellates from *T. granulosa* were few in number (2 to 7 per thin film) and all flagellates were similar in size and shape. Study showed the parasites to be identical with the small, slender forms of *T. ambystomae*. On the basis of morphological similarities, then, it appears that, aside from *Ambystoma gracile*, *Taricha granulosa* may be considered an autochthonous host of *T. ambystomae*.

While studying stained preparations of *T. ambystomae* from one *A. gracile* a new morphological type of the parasite was encountered. Aside from large, broad individuals a few distinct forms were noted. They were roughly rounded to elliptical in shape, averaging 29.0 microns in length by 16 microns in width; one end (the anterior) was semicircular, the posterior bluntly rounded. The nucleus was centrally located and elliptical in shape; no apparent endosome was seen. The rod-shaped kinetoplast was at the periphery, some 7 microns from the posterior end. The axoneme, arising from the kinetoplast, bordered a narrow undulating membrane that extended across the body. The undulating membrane continued around the anterior, rounded end and then progressed posteriorly and terminated a short distance from its origin. There was no free flagellum. The cytoplasm had no granules and its coloration, as well as that of the nucleus and kinetoplast, resembled that of the previously described large, broad forms. The gross morphology of the parasite resembled that of certain forms of *T. rotatorium*.

The phenomenon described by Barrow (1954, Tr. Am. Mic. Soc. **73**: 242–257) whereby the mechanism of preparing thin smears tends to distort the width of *Trypanosoma diemyctyli*, does not appear to be applicable in this instance. The rounded-elliptical body shape, want of pointed extremities, course of the undulating membrane and lack of tears or lines of stress seem to indicate that these bodies are natural stages in the vertebrate cycle of *T. ambystomae*. —Donald L. Lehmann, *Pacific Marine Station, Dillon Beach, Calif.*

A CASE OF CUTANEOUS MYIASIS IN A CHILD CAUSED BY *MUSCINA* SP.

Muscina species have rarely been taken from the skin of living mammals, although there are records from the alimentary canal (M. T. James, 1947, U. S. Dept. Agric. Misc. Publ. 631). Carter and Blacklock (1913, Brit. Med. Journ. **1**: 72) found *Muscina stabulans* larvae associated with those of *Calliphora vicina* (syn. *C. erythocephala*) and *Fannia canicularis* in a case of external myiasis of a monkey. Haddow and Thomson (1937; Parasitology **29**: 96–116) found single specimens of *Muscina pabulorum* involved in sheep strike in Scotland; in two cases they were associated with *Phaenicia sericata* (syn. *Lucilia sericata*) and in one with *Protophormia terraenovae*, presumably as well as with *P. sericata*. MacLeod (1943, Bull. Entom. Research **34**: 65–88) found *Muscina pabulorum* involved in two cases of myiasis of hogs and once in sheep myiasis; in all three cases the larvae were associated with those of *Phaenicia sericata* and in one case *Lucilia caesar* larvae were also present. In all of these reports of external myiasis due to *Muscina* larvae, a more aggressive species was also present.

Dr. H. A. Wente of Rochester, Minnesota submitted a maggot to the authors for identification, and subsequently the following case history was obtained (October 26, 1954):

"G., age 9, (a resident of Elgin, Minnesota) developed a 'lump' on the flexor surface of the right wrist. It was very painful and she wanted her mother to 'open it.' The child stated that she thought there was a thistle in it. The lump, as she described it, was reddened, elevated, and had no external opening. The mother took a needle and attempted to open it without success. She drew a little blood, but there was no pus. She then soaked it in hot water two or three times in the next ten hours and placed a vaseline bandage on the area. When she removed the bandage twelve hours after the original attempt to open it, she found this 'worm,' as she described it, protruding from the open lesion. She (the child) had several other lesions on the arm and in the same location. These were smaller and receded after this period. She

came in to see me the morning after finding the larva and I suggested hot packs to the original lesion. It improved immediately.

"Now G. has a slightly depressed scar about the size of a thumb tack head on the flexor surface of the right wrist. The child has not been out of the county and lives under poor hygienic conditions. From the mother's history, it seems that this lesion was not open prior to the time the larva appeared. The child is in good health now and I did not do laboratory studies on her."

The larva was thought to be a third instar *Muscina assimilis* and was therefore submitted to Dr. M. T. James of the State College of Washington and Drs. C. W. Sabrosky and W. W. Wirth of the United States National Museum for confirmation; all three agree that it is *Muscina* sp., probably *assimilis* (Fallin). The specimen has been deposited in the United States National Museum.

This appears to be the first record of *Muscina* sp. alone causing cutaneous myiasis in a living animal.

(Paper No. 3319 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.)—A. RALPH BARR, *Department of Entomology and Economic Zoology, University of Minnesota, and* JOHN H. THOMPSON, JR., *Sections of Clinical Pathology and Biochemistry, Mayo Clinic, Rochester, Minnesota.*

"AEROSOL OT 100%," A WETTING AGENT FOR MOLLUSCACIDES

In the course of testing molluscacides against two intermediate host snails of the deer liver fluke, *Fascioloides magna*, the insolubility of many of the organic chemicals led to a search for a suitable wetting agent. Of ten wetting agents and detergents tested, Aerosol OT 100%, Sterox AJ, Sterox CD, Sterox SK, Tergitol 7, Tergitol P-28, Fab, Vel, Kleer-Mor, and Joy, the most useful in this laboratory was Aerosol OT 100%, a product of the American Cyanamid Company.

One milliliter quantities of the compound in 1:1000 and in 3 percent concentrations effectively wetted one gram amounts of the test chemicals. The test chemicals included copper pentachlorophenate, dinitro-o-cyclohexyl-phenol (DCHP), DN-1 (a mixture containing 40 percent DCHP), 2,4-dinitro-6-phenylphenol, and 2-cyclohexyl-4, 6-dinitrophenol dicyclohexylamine salt. Paper NS 169, published with the approval of the director of the Wisconsin Agricultural Experiment Station and supported in part by Pittman-Robertson Project (W-15-R) of the Wisconsin Conservation Department.—F. R. JOHNSON AND A. C. TODD, *Department of Veterinary Science, The University of Wisconsin.*

LARVAL NEMATODE RECORDED FROM HONEYBEE *APIS MELLIFERA* L.

During the summer of 1954 two larval specimens of *Agamomermis sp.* were found while making examinations of 2111 honeybees (*Apis mellifera* L.) in the Ithaca, New York area.

These honeybees were taken in the field while making a study of competition between honey plants in the area. The bees were taken from three stations about five miles apart, extending in a straight line essentially north and south, east of Ithaca. The parasites were found in the middle station only, which was near the Ithaca airport where only 294 bees were taken. The 2111 bees were collected from 11 flowering plants between June 7 and August 18, 1954. To determine the effects of competition the bees were collected in cyanide jars. The heads were removed and the contents of the abdomen removed in order to retrieve the honey stomach. The parasites were found in the abdomen, nearly filling it. The method was such that no parasites could have remained undetected.

The first parasite, taken on July 5 from a bee working birdsfoot trefoil (*Lotus corniculatus* L.) was 9.0 cm. long and 0.292 mm. in diameter. This bee had only about three to five mg. of nectar in the honey stomach, which is considered a very small load. The second parasite was taken on July 12, from a bee working on a milkweed (*Asclepias syriaca* L.). This bee was carrying a medium size load of nectar weighing about 12 to 15 mg. This parasite was 7.5 cm. long and 0.277 mm. in diameter.—ROGER A. MORSE, *State Plant Board of Florida, Gainesville, Florida.*

THE AMERICAN JOURNAL OF TROPICAL MEDICINE

Official Organ of the American Society of Tropical Medicine

L. W. HACKETT, Editor

Presents clinical, laboratory and environmental studies on diseases, their causative agents and vectors from the viewpoint of tropical public health.

Published bi-monthly, one volume a year.

Subscription price per volume $6.00 (plus 50 cents postage outside the Postal Union)

First published 1921

THE WILLIAMS & WILKINS COMPANY

Mt. Royal and Guilford Aves. Baltimore 2, Md.

The Journal of Protozoology

Published quarterly by the Society of Protozoologists

Volume 2 current in 1955

The JOURNAL OF PROTOZOOLOGY is designed for the publication of the results of original work concerned with any aspect of the study of protozoa. The journal provides a common medium for the taxonomist, the physiologist, the parasitologist, and the biochemist, working with protozoan material.

Editor: William Trager
The Rockefeller Institute for Medical Research
66th St. and York Avenue, New York 21, N. Y.

Subscription price is $9.00 per year, except for graduate students, for whom it is $6.00. Inquiries regarding subscriptions and membership in the Society of Protozoologists should be addressed to Norman D. Levine, Secretary, College of Veterinary Medicine, University of Illinois, Urbana, Illinois.

BIOLOGICAL ABSTRACTS

Covers the world's biological literature

How do you keep abreast of the literature in your field? Perhaps some relatively obscure journal has published a revealing paper on the very subject in which you are most interested. Informative, concise abridgments of all the significant contributions will be found in *Biological Abstracts.*

As well as the complete edition, covering all fields of biology, *Biological Abstracts* also is published in five low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields. Section C—Abstracts of Microbiology, Immunology, Public Health, and Parasitology—affords a very complete coverage of the Parasitologists' literature. The price is only $6.00 a year (Foreign, $6.50).

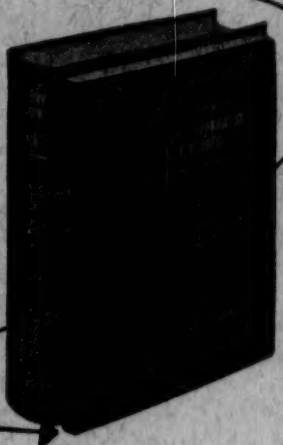
Write for a sample copy.

BIOLOGICAL ABSTRACTS

University of Pennsylvania
Philadelphia 4, Pa.

Now being distributed . . .

SUPPLEMENT
to the
THOMAS CATALOGUE



1600 New Items
Current Price List

NOW . . . 22,000 items offered to you for immediate shipment from our stock.

This new Supplement follows the tradition of the Thomas catalogue on which the following comment was made by a leading scientific periodical:

"More than a catalogue . . . a source of reference the user will place on his shelves with manuals and textbooks."

The encyclopedic catalogue of 1472 pages and the new 264-page supplement list a total of approximately 22,000 current items, adequate stocks of which are carried for immediate shipment.

The selection of apparatus is based upon more than half a century of experience in supplying the needs of laboratories of Chemistry and Biology, both large and small. Choice was made after careful consideration, and testing by the Thomas technological staff. Items included are therefore offered with assurance of being satisfactory for the purpose intended.

This new Supplement with current Price List, in combination with the general catalogue, constitutes an up-to-date listing of one of the most comprehensive assortments of apparatus and reagents available from any single source.

SUPPLEMENT

Detailed and factual listings, fully indexed, of new items added to our stock since 1950.

Complete "Price List" of current apparatus items in *both* catalogue and supplement.

Supplement now being mailed to all who have the Thomas catalogue.

SYMBOL OF QUALITY
A.H.T.CO.
LABORATORY APPARATUS

ARTHUR H. THOMAS COMPANY

Laboratory Apparatus and Reagents

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA.

Teletype Services: Western Union WUX and Bell System PH-72

CONTENTS

JOURNAL OF PARASITOLOGY

Volume 41, Number 5 **October, 1955**

THE MORPHOLOGY AND LIFE HISTORY OF *Amphiacantha ovalis* AND *A. attenuata*. TWO NEW HAPLOSPORIDIAN PARASITES OF GREGARINES. **Joseph W. Stubblefield** (443–459)

NOTES ON THE MORPHOLOGY AND HOST-PARASITE SPECIFICITY OF *Fibricola cratera* (BARKER AND NOLL, 1915) DUBOIS 1932 (TREMATODA: DIPLOSTOMATIDAE) **Martin J. Ulmer** (460–466)

SUSCEPTIBILITY STUDIES IN SCHISTOSOMIASIS. IV. SUSCEPTIBILITY OF WILD MAMMALS TO INFECTION BY *Schistosoma haematobium* IN EGYPT, WITH EMPHASIS ON RODENTS. **Robert E. Kuntz and George M. Malakatis** (467–475)

THE USE OF AN ACID SOLUBLE PROTEIN FRACTION IN THE FLOCCULATION TEST FOR THE DIAGNOSIS OF TRICHINOSIS. **Elvio H. Sadun and L. Norman** (476–482)

THE ANTIGENIC ROLE OF THE EXCRETIONS AND SECRETIONS OF *Trichinella spiralis* IN THE PRODUCTION OF IMMUNITY IN MICE. **Charles H. Campbell** (483–491)

THE TAXONOMY AND BIOLOGY OF *Epitedia wenmanni* (ROTHSCHILD, 1904) AND *E. testor* (ROTHSCHILD, 1915) (HYSTRICHOPSYLLIDAE: SIPHONAPTERA). **Allen H. Benton** (491–495)

THE HELMINTH FAUNA OF NICARAGUA I. A NEW GENUS AND SPECIES OF CESTODE (*Dilepidinae*) FROM THE HUMMINGBIRD, *Phaeochroa cuvierii roberti*. **Kenneth A. Neiland** (495–498)

EFFECTS OF CORTISONE ON *Trichinella spiralis* INFECTIONS IN NON-IMMUNIZED MICE. **Coit M. Cker** (498–504)

STUDIES ON *Trichostrongylus axei* (COBBOLD, 1879) I. SOME EXPERIMENTAL HOST RELATIONSHIPS. **J. H. Drudge, S. E. Leland, Jr., Zae N. Wyant, and G. W. Elam** (505–511)

MYXOSPORIDIA IN FISH IN WATERS EMPTYING INTO GULF OF MEXICO. **R. H. Rigdon and John W. Hendricks** (511–518)

Steptolaelaps (ACARINA: LAELAPTIDAE) A NEW GENUS OF MITES PARASITIC ON NEOTROPICAL RODENTS. **Deane P. Furman** (519–525)

THE ESTABLISHMENT OF A STRAIN OF *Australorbis glabratus* WHICH COMBINES ALBINISM AND HIGH SUSCEPTIBILITY TO INFECTION WITH *Schistosoma mansoni*. **Walter L. Newton** (526–528)

THE DISTRIBUTION OF LARVAL STAGES OF *Aspiculuris tetraptera* IN THE INTESTINE OF MICE. **K. F. Chan** (529–532)

NOTES ON THE GENUS *Maritrema* NICOLL, 1907 (TREMATODA: MICROPHALLIDAE) WITH THE DESCRIPTION OF TWO NEW SPECIES. **William H. Coil** (533–537)

AN UNUSUAL CASE OF SCHISTOSOMIASIS WITH SPECIAL EPIDEMIOLOGICAL ASPECTS. **Emmanuel Margolis** (538–541)

THE EFFECT OF DIET ON THE LENGTH OF THIRD STAGE LARVAE PRODUCED BY ADULT *Haemonchus contortus* HARBORED BY LAMBS. **R. F. Shumard, C. A. Herrick and A. L. Pope** (542–544)

TOXOPLASMA IN TISSUES OF MAN AND PETS. **A. C. Krause** (545–548)

PHYSICAL OBSERVATIONS ON ECHINOCOCCUS EGGS, PART I. **H. F. Meyers** (548–551)

RESEARCH NOTES:

A NEW HOST RECORD FOR *Trypanosoma ambystomae* WITH THE DESCRIPTION OF AN ADDITIONAL MORPHOLOGICAL TYPE OF THE PARASITE: **Donald L. Lehmann.** A CASE OF CUTANEOUS MYIASIS IN A CHILD CAUSED BY *Muscina* SP.: **A. Ralph Barr and John H. Thompson, Jr.** "AEROSOL OT 100%," A WETTING AGENT FOR MOLLUSCACIDES: **F. R. Johnson and A. C. Todd.** LARVAL NEMATODE RECORDED FROM HONEYBEE *Apis mellifera* L.: **Roger A. Morse.** (552–553)

BUSINESS PRESS, INC.
LANCASTER, PA.